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The Physiology of Salt Tolerance in Crop Plants

Conducted by
C.G. Suhayda, D.L. Ehret,
R.E. Redmann and B.L. Harvey

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THE PHYSIOLOGY OF SALT TOLERANCE
IN CROP PLANTS

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ABSTRACT

This is the final report on research funded from a Canada-Saskatchewan ERDA agreement with the primary objective of identifying key physiological processes controlling salt tolerance in crop species. Plant stresses from soil salinity in Saskatchewan arise from excess quantities of sodium and magnesium sulfates. The physiological effects of these salts have not been studied to the same extent as in saline soils where chloride salts are predominant.

Early results from this project demonstrated a strong interaction between calcium supply and the growth response of barley and wheat cultivars to salinity stress. Increases in the calcium:total cation ratio (Ca/TC) of saline solution cultures with calcium addition ameliorated the adverse effects of salinity on plant growth, but more so in wheat than barley.

The important role played by calcium in mediating the salt tolerance response of barley species was further characterized in a series of comprehensive solution culture experiments conducted in controlled environmental chambers. The growth of Harrington and Bonanza barley plants showed a strong dependence upon the Ca/TC and improved substantially when plants were salinized at the higher Ca/TC in the solution cultures. In sharp contrast, foxtail barley, a native halophyte, displayed substantial salt tolerance even under conditions of a limiting calcium supply. On the whole plant level, the greater salt tolerance of *H. jubatum* plants appears to be correlated with the ability of this species to maintain adequate root growth despite harsh stress conditions. Analysis of root and

shoot tissue ion contents revealed that the wild barley species had superior ion selectivity and ion transport properties as compared to the commercial varieties.

We selected *H. jubatum* for the purpose of using it as a model system to examine how a salt tolerant, wild barley species copes with salinity stress. The superior ion selectivity and ion exclusion properties of this plant are likely based upon transport processes at the level of root tissue membranes. We believe that in *H. jubatum* the root serves as the primary barrier to salt stress and thus mediates the salt tolerance response at the whole plant level. This wild species appears to be a good source of salt tolerant germplasm. One of the challenges for future research in this area will be the identification, and selective transfer of those genes conferring salt tolerance in foxtail barley into commercial barley lines.

At the cellular level salt-induced membrane damage resulted in the leakage of cell contents into the solutions bathing the tissue. Membrane leakage tests showed that calcium amendments reduced leakage, presumably by altering membrane permeability, which also resulted in the improved root growth of barley seedlings. Leakage studies of leaf, coleoptile, and root tissues from a series of barley varieties subjected to saline media varied with experimental conditions, and therefore did not provide a consistent rating of relative salt tolerance. Subsequently, additional membrane studies using a system of plasma membrane vesicles isolated from salinized Harrington roots revealed that prolonged salinity stress alters membrane properties that are fundamental to ion transport. The calcium supply as expressed by the Ca/TC had a strong mediating affect on membrane properties. A sodium extrusion mechanism appears to be present brsent on the Harrington root cell plasma membrane.

In the final year of the project we initiated experiments to determine the role and possible interaction of calcium nutrition and soil salinity on the yield and physiology of field grown barley. Calcium amendments increased the level of soil calcium, and improved the ion relations of plant tissues. Treatments adversely affected vegetative growth while leaving grain yield unaffected. We believe that these latter affects may be linked to the form of calcium used in the amendment added, and possibly to the overriding effects of other stresses in the field situation. These preliminary conclusions remain to be verified in future field studies.

The results of this study have provided a framework for a new course of research which seeks to identify and characterize physiological and cellular mechanisms that may be responsible for the observed differences in salinity tolerance between wild species ecotypes and commercial barley lines. A greater emphasis will be placed upon the characterization of salinity-induced changes in the functional and structural properties of membranes prepared from barley species and the influence of calcium. A closely associated parallel investigation was recently initiated to examine the production of stress proteins produced in response to salinity.

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1.0 INTRODUCTION

1.1 Primary Objective

This is the final report of a project carried out under an agreement between Saskatchewan Agriculture and the University of Saskatchewan, funded from the Canada-Saskatchewan ERDA Agreement. The primary objective was to investigate processes of salinity tolerance in crops, and in particular the physiological characteristics which control salt tolerance. Furthermore, the research was planned so as to identify physiological properties of plants which could in the future be used to select for improved cereal crop varieties.

1.2 Background and Specific Objectives

Extensive areas of Saskatchewan have soils containing concentrations of soluble salts sufficient to adversely affect plant growth, and make these areas unsuitable for conventional crop production (Dodd *et al.* 1964, McKell *et al.* 1986, Henry 1987). On a global scale, soil salinity affects 400 to 950 million hectares (Epstein *et al.* 1980). For the most part, salinization is a natural geological process but in some areas land use practices such as summer fallowing and irrigation also contribute to the problem (McKell *et al.* 1986).

Virtually all plants of agronomic and horticultural importance are adversely affected in growth and yield by salinity stress (Holm 1983, Maas 1986). If the salt tolerance of crop plants could be enhanced the resulting benefits could be greater diversity of crops under cultivation, higher productivity on salinized lands, and reduced costs for reclamation of saline soils.

Our work was focused on the improvement of the salt tolerance of barley, a prominent grain crop of the prairie provinces. The identification of salt tolerance mechanisms in related wild barley species and other grasses that allow these plants to thrive in harsh saline environments, combined with conventional and novel genetic selection techniques could enable the eventual transfer of the desired salt tolerance traits to commercial lines. Much of the earlier research on barley salt tolerance has concentrated on sodium chloride salinity, whereas our research is focussed on sulfates of sodium and magnesium, which are the most significant salts in Saskatchewan soils (Saskatchewan Soil Survey 1984).

The specific goals of the research described here were 1) to identify physiological parameters for use in a screening program to determine the relative salt tolerances of commercial barley varieties (*Hordeum vulgare*) and related salt tolerant wild species (*Hordeum jubatum*), 2) to characterize the protective effect of calcium amendments on plant growth under saline conditions especially on relative yield and ion distribution in roots, stems and leaves and 3) to elucidate the biochemical and physiological mechanisms used by commercial varieties and wild barley species to cope with specific ion stresses, focusing mainly on membrane-mediated ion transport processes and the influence of calcium ions on these processes.

Our interest in membrane properties stems from the fact that membranes provide the barriers for the exclusion of, or the selective uptake of ions into the cell and within the cell provide the basis for the compartments used to sequester ions. Eventually, using this approach, we hope to identify salt tolerant germplasm, reliable physiological or biochemical membrane markers that can be used in a rapid screening test for salt tolerance, and cultivars that efficiently use calcium as a component of salt tolerance mechanisms.

1.3 Organization of Report

The results of the research have been arranged in a chapter format. One of the major sections is Chapter 2, which describes initial experiments showing the key role of calcium nutrition in relation to salinity stress in crops. This important early finding formed a foundation on which much of the following work was based.

In Chapter 3 we describe a comprehensive set of experiments demonstrating that salt effects on barley can be ameliorated by addition of calcium to solution culture. This work also demonstrates the genetic potential for extreme salt tolerance which exists in wild barley, a native halophyte in Saskatchewan. The locus for the most severe salt effects is the root system.

Ultimately is is clear that excess salts in the plant environment adversely impact on the membranes of individual cells, paricularily in the roots, which are the first "line-of-defense" against soil salinity. The research described in Chapter 4 shows how certain key membrane properties are affected by salts, and also demonstrates that differences between barley and the native halophyte wild barley occur at the membrane level as well as the whole plant level.

Chapter 5 describes the results of a series of experiments on the effects of salinity and calcium supply on the growth and membrane integrity of young barley seedlings.

The importance of calcium nutrition in relation to salt tolerance led to the initiation of preliminary work on the effect of calcium soil amendments on growth, development and tissue ion content of barley growing under field conditions. Chapter 7 describes the results of attempts to use a physiological measure called the membrane leakage test to identify differences in the responses of barley varieties to a standard salt treatment.

The report concludes with a chapter on overall conclusions, and suggestions for areas of future research.

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2.0 SALINITY-INDUCED CALCIUM DEFICIENCIES IN WHEAT AND BARLEY

2.1 Introduction

Despite a tendency for salt exclusion, the mineral nutrition of non-halophytes is influenced by the presence of salt, often as a consequence of ion interactions. For example, Na-K and Cl-NO_3 interactions are well documented (Greenway and Munns, 1980). However, it is generally believed that ion excess in the tissue rather than nutrient deficiency (for example, Na toxicity rather than K deficiency) is the causative agent in reducing growth through specific ion effects.

In the case of Ca, however, recent evidence suggests that yield loss is more consistently related to low Ca content of the tissue than to high Na content (Janzen and Chang, 1987). Calcium is known to alleviate the effects of salt, possibly by maintenance of ion selectivity of membranes (Greenway and Munns, 1980). Further, evidence of Ca/cation interactions (Cramer et al., 1985) suggests that Ca/cation ratios may be more important than absolute Ca concentration in influencing plant response to salinity.

Saline soils of the Canadian prairies are often dominated by Na and Mg sulfates (Fowler and Hamm, 1980), which when compared to NaCl, may exert a more severe reduction in growth (Strogonov, 1964). High sulfate levels may decrease available Ca through precipitation reactions, which may, in part, be responsible for reduced Ca levels in cereals on these soils (Janzen and Chang, 1987).

In view of the relatively meager information on crop response to this type of salinity, two species differing in salt tolerance were compared in solution cultures which were typical in composition of sulfate-dominated dryland saline soils. Plant response to Ca was determined and related to the salinity tolerance of each crop.

2.2 Methods

Seeds of wheat (cv. Neepawa) and barley (cv. Abee) were germinated in water on filter paper and transferred to vermiculite-filled plastic beakers (one seedling each) having highly perforated bottoms. The beakers were mounted in a platform suspended above containers of nutrient solution each with a rooting volume of nine plants per 13.5 L of solution. Wheat and barley were grown in separate containers except in Expt. 1A, where 8 plants of each species were grown together in 10 L containers. Solutions were aerated continuously and pH was adjusted to 6.5 every other day with H_2SO_4. Solutions were topped up with water daily, and were replaced once a week. The base (non-saline) solution was made up in deionized water, and was composed of, in m$\underline{M}$, 0.2 NH_4, 2.0 Mg, 2.5 NO_3, 1.5 K, 2.75 SO_4, 0.2 PO_4, 2.5 to 4.0 Ca (concentration varied slightly with experiment), 3.0 Cl, and in µ$\underline{M}$, 50 Fe (as Fe-EDDHA), 6.0 B, 0.8 Mn, 0.5 Zn, 0.15 Cu, and 0.15 Mo. Manganese toxicity was observed in preliminary experiments, and silicate (as Na_2SiO_3 at 26 µ$\underline{M}$) was routinely added to alleviate this problem (Lewin and Reimann, 1969).

Salinity was imposed by the addition of Na_2SO_4, $MgSO_4$, $CaSO_4$, and $CaCl_2$ to the base solution. Sodium, Mg, SO_4, and Cl concentrations were typical of moderately to severely saline soils, according to the classification of Fowler and Hamm (1980) based on the saturated paste extraction method. Calcium concentrations varied according to treatment and experiment. With the exception of $CaSO_4$, salts were added gradually in increments of 10 to 15 m$\underline{M}$ d^{-1} of total salt. Because of its relatively poor solubility, $CaSO_4$ was dissolved overnight in fresh nutrient solution, to which the other salts were then added. Final salt concentrations were maintained at a constant value through the duration of the treatment period. Unless otherwise stated, treatments were started 10 days after seeding, and continued for 21 days. Treatment solution water potentials were determined with thermocouple psychrometers (J.R.D. Merrill) and the mineral content of solutions was verified with plasma emission spectroscopy. All cation ratios are expressed on a molar basis.

Plants were maintained in a growth chamber (Conviron E-15) with 24°C day/18°C night ramped temperature, an irradiance of 400 μ mol m^{-2} s^{-2} PPFD for 14.5 h d^{-1}, and except in one humidity experiment, a vapour pressure deficit (vpd) of 1.5-1.8 kPa day/0.8-1.0 kPa night (40-50% day/50-60% night rh). Containers of plants were rotated periodically within the growing space to ensure uniformity. Plants were rinsed at the crown with deionized water every other day to prevent salt accumulation.

A series of five experiments was conducted. Each experiment was repeated once, sometimes with a few minor variations. The first experiment tested the effects of a combination of Na_2SO_4 plus $MgSO_4$ salinity on wheat and barley growth, and the effects of Ca on the response to salinity. Plants were grown in either a non-saline solution, or one of two saline solutions which differed in Ca concentration and consequently in the Ca/(Na + Mg) ratio. For convenience, the two saline treatments were termed "saline" (low Ca) or "amended saline" (high Ca). In trial A, the Ca concentrations of the non-saline and saline solutions were the same (3.5 m$\underline{M}$) and representative of saturation extracts from non-saline soils (Fowler and Hamm, 1980). The Ca concentration of the amended saline treatment was 10 m$\underline{M}$, typical of saturation extracts of saline soils (Fowler and Hamm, 1980). In trial B, the Ca concentrations of the saline treatment was 9 m$\underline{M}$, and that of the amended saline treatment 18 m$\underline{M}$, the latter being approximately twice the concentration found in saturation extracts of saline soils. Experiment 2 was similar, except that $MgSO_4$ alone replaced the Na_2SO_4 plus $MgSO_4$ combination. Experiment 3 examined the effects of the Ca/(Na + Mg) ratio on wheat and barley growth under non-saline conditions. Plants were grown in the base solution with low concentrations of Na, Mg and Ca adjusted to the appropriate Ca/(Na + Mg) ratio.

Since wheat showed a greater response to Ca than did barley under saline conditions, further work was conducted to characterize the nature of the response in wheat. The effect of night-time humidity on the response to salinity and Ca was examined in Experiment 4. Groups of plants were grown in non saline, saline or amended saline solutions as in Experiment 1, and either enclosed at night in plastic lined with damp paper towelling, or left at ambient humidity. Humidities were maintained at vpd's of either 0.2-0.1 kPa (90-95% rh) or 0.8-1.0 kPa (50-60% rh). Humidity was measured with an aspirated wet-bulb, dry-bulb thermometer. In Experiment 5, the effects of salinity and Ca on leakage of UV-absorbing substances from wheat leaf tissue was examined. Plants were grown in non saline, saline or amended saline solutions as described in Experiment 1. Twenty leaf sections, each 2 cm in length, were cut from leaves on the main shoot of plants from each treatment. After rinsing for 1 h in distilled water, the samples were drained and shaken in 25 ml distilled water at 25°C for 24h. Absorbance at 280 nm (A) was then determined for each solution. The samples were frozen at -70°C for at least 4h, allowed to thaw, and shaken for 2-3h. Absorbance was again determined at 280 nm (A'). The relative leakage ratio (RLR) was calculated as $RLR=A/A'$.

On termination of each experiment (except 5), leaf area (lamina only) was determined for each plant with a leaf area meter (Li-Cor LI-3000). In Experiment 1, 2 and 3, stem (actually overlapping leaf sheaths), leaf, and occasionally root dry weight was also determined for each plant after oven drying.

In some cases the mineral content of the dried leaf tissue was measured. Since it was not feasible to analyse each replicate separately, the tissue of each plant was ground and pooled within each treatment. In one instance (Experiment 1) the mineral content of very young leaves was also determined. Here, the stem (leaf sheaths) was cut open and both the youngest emerging and oldest unemerged leaves were withdrawn. Samples were then pooled for analysis. After nitric-perchloric acid digestion, samples were analysed using plasma emission spectroscopy. All solution and tissue mineral analyses were conducted by the Saskatchewan Soil Testing Laboratory. Variance in the mineral content was estimated using mean values obtained from repeated experiments.

Statistical analysis of the tabulated data was performed using analysis of variance (ANOVA). Variance in the percent reduction in leaf area or dry weight due to salinity was calculated after values for each replicate (plant) in the saline treatment were converted to percentages of the mean value of the non-saline treatment; standard error was then calculated for the mean percent leaf area or dry weight reduction.

2.3 Results

At the relatively low Ca concentration of 3.5 mM, the growth of both species was reduced by salt (Table 2.1, A). Wheat showed a 68 ± 2 percent reduction in leaf area compared to 42 ± 3 percent in barley; plant dry weight was reduced 44 ± 3 percent in wheat and 28 ± 3 percent in barley (means of 16 plants ± s.e.). The Ca/(Na + Mg) ratio in this saline treatment was 0.06. The amended saline treatment containing 10 mM Ca increased the Ca/(Na + Mg) ratio to 0.17. Compared to the non-amended saline treatment, leaf area was increased 138 percent in wheat and 25 percent in barley. Plant dry weight increased 42 percent in wheat but was not affected in barley.

The experiment was repeated at similar Ca/(Na + Mg) ratios to those just described, but at higher salt and Ca concentrations (Table 2.1, B). In the saline treatment (9 mM Ca, Ca/(Na + Mg) ratio of 0.08), leaf area was reduced 52 ± 3 percent in wheat and 17 ± 3 percent in barley; dry weight was reduced 34 ± 3 percent and 11 ± 4 percent in wheat and barley, respectively (means of 9 plants ± s.e.). An increase in the Ca/(Na + Mg) ratio to 0.17, by increasing the Ca concentration to 18 mM, improved the leaf area of wheat by 47 percent and the dry weight by 23 percent when compared to the non-amended salt solutions. This increase in growth was apparent despite a small decrease in the solution water potential (-30, -350, and -380 kPa in non-saline, saline, and amended saline treatments, respectively). The Ca amendment, however, had no significant effect on barley growth. These trends were also apparent in the number of visibly Ca-deficient leaves. Young emerging leaves of tillers, and to a lesser extent, the main shoot, showed collapse and necrosis of the distal portion. Symptoms were often evident in the early stages of emergence, with subsequent growth being normal. Even so,

Table 2.1 Effect of Na and $MgSO_4$-salinity and Ca on leaf area and plant dry weight of wheat and barley. Values are the means of 16 plants (A) or 9 plants (B). Experiment 1.

Treatment				Wheat[a]		Barley[a]	
	Na	Mg	Ca	Leaf Area	Plant Dry Weight	Leaf Area	Plant Dry Weight
	(m$\underline{M}$)			(cm^2)	(g)	(cm^2)	(g)
A.							
Non-saline	<0.1	2	3.5	268a	1.27a	571a	3.11a
Saline	30	30	3.5	85c	0.71c	329c	2.24b
Amended Saline	30	30	10.0	202b	1.01b	412b	2.35b
B.							
Non-saline	<0.1	2	3.5	409a	2.79a	501a	4.18a
Saline	45	60	9.0	195c	1.84c	414b	3.71b
Amended Saline	45	60	18.0	286b	2.26b	422b	3.87b

[a] Mean separation within columns for each experiment by Duncan's multiple range test, 5% level.

substantial loss of area was evident in these leaves. Neither species showed Ca deficiencies under non-saline conditions. Wheat had a greater number of Ca-deficient leaves than barley in the presence of salt and Ca amendments reduced the number of Ca-deficient leaves from 14.9 ± 0.7 to 4.7 ± 0.6 per plant in wheat and from 3.3 ± 0.8 to 0 ± 0 per plant in barley (means of 9 plants ± s.e.).

Under saline conditions, the Ca content of the leaves decreased, particularly in barley (Table 2.2). Sodium and Mg content increased with salinity in both species, with Mg being the highest in wheat and Na being the highest in barley. Amendment of the salt solution with additional Ca significantly increased Ca in barley and reduced Mg in wheat. Sodium was not affected in either species. The leaf Ca/(Na + Mg) ratio was reduced by salinity, more so in wheat than in barley. Calcium amendment increased this ratio in wheat but not in barley.

Salinity and Ca also influenced the mineral status of young emergent leaves of wheat (barley was not measured). The Ca/(Na + Mg) ratios of the youngest emerging plus oldest unemerged leaves were 0.23 ± 0.01, 0.05 ± 0.005 and 0.09 ± 0.01 in non-saline, saline and amended saline treatments, respectively (values are means of three trials, each with 9 plants, ± s.e.).

Plant response to $MgSO_4$ alone (Experiment 2) was similar to that of a combination of $MgSO_4$ and Na_2SO_4. In the first trial (Table 2.3, A), salinity (4 mM Ca, Ca/Mg ratio of 0.13) reduced the leaf area of wheat 32 ± 2 percent and that of barley 19 ± 4 percent; shoot dry weight was reduced 29 ± 2 percent in wheat and was not significantly affected in barley (means of 16 plants ± s.e.). Amendment of the saline solution with Ca (10 mM, Ca/Mg ratio of 0.31) increased wheat leaf area 24 percent and dry weight 12 percent compared to the non-amended treatment. There was no significant effect of the

Table 2.2 **Effect of Na and $MgSO_4$-salinity on cation content of wheat and barley leaves.**

Species	Treatment	Ca/(Na + Mg)[b]	Leaf[a] Na	Mg	Ca	Ca/(Na + Mg)
			(µmol gm^{-1} dry weight)			
Wheat	Non-Saline	1.50	7d	128d	177a	1.30a
	Saline	0.08	130b	447a	91b	0.16c
	Amended Saline	0.18	106bc	315b	117b	0.28b
Barley	Non-Saline	1.50	23cd	116d	177a	1.30a
	Saline	0.08	453a	224c	49c	0.08d
	Amended Saline	0.18	477a	204cd	89b	0.13cd

[a] Mean separation within columns by Duncan's multiple range test, 5% level. Each value is the mean of 8, 8 and 7 replicates in wheat and 4, 4 and 3 replicates in barley in non-saline, saline and amended saline treatments, respectively. Each replicate consisted of at least 9 plants.

[b] Average of values ranging from 1.25 - 1.75, 0.06 - 0.09, and 0.17 - 0.20 in non-saline, saline and amended saline treatments respectively.

Table 2.3 Effect of $MgSO_4$-salinity and Ca on leaf area and shoot dry weight per plant of wheat and barley. Values are the means of 16 plants. Experiment 2.

Treatment	Mg (mM)	Ca (mM)	Wheat[a] Leaf Area (cm^2)	Wheat[a] Shoot Dry Weight (g)	Barley[a] Leaf Area (cm^2)	Barley[a] Shoot Dry Weight (g)
A.						
Non-saline	2	4	272a	1.21a	405a	1.82a
Saline	32	4	184c	0.86c	330b	1.65a
Amended saline	32	10	229b	0.96b	352ab	1.79a
B.						
Non-saline	2	3	294a	1.20a	479a	2.15a
Saline	40	8	224b	0.98c	422b	1.85b
Amended saline	40	15	278a	1.11b	391b	1.90ab

[a] Mean separation within columns for each experiment by Duncan's multiple range test, 5% level.

Ca amendment on barley leaf area or dry weight. The second trial (Table 2.3, B), conducted at somewhat higher salt and Ca concentrations, produced similar results. Salinity (8 mM Ca, Ca/Mg ratio of 0.2) reduced wheat leaf area 24 ± 2 percent and barley leaf area 12 ± 4 percent; dry weight was reduced 18 ± 3 percent in wheat and 14 ± 5 percent in barley (means of 16 plants ± s.e.). Again, Ca amendment (15 mM Ca, Ca/Mg ratio of 0.38) increased wheat leaf area 24 percent over non-amended plants, but had no significant effect on barley. Dry weight increased 13 percent in wheat but was not affected in barley.

Neither species showed Ca deficiency symptoms under non-saline conditions, but both showed symptoms in the saline treatments. In the second trial, for example, salinity produced 4.3 ± 0.3 Ca-deficient leaves per plant in wheat and 1.1 ± 0.3 in barley; amendment with Ca reduced the number of deficient leaves to 0.1 ± 0.1 in both species (means of 16 plants ± s.e.).

In the absence of salinity and at an average solution water potential of -100 kPa (Experiment 3), leaf area and shoot dry weight decreased more in barley than in wheat with decreasing Ca/(Na + Mg) ratio of the solution. In the first trial (Table 2.4, A) of four Ca/(Na + Mg) ratios (1.5, 0.4, 0.2 and 0.06), barley leaf area was significantly reduced at Ca/(Na + Mg) ratios of 0.2 and 0.06 (11 percent and 24 percent reductions, respectively). Wheat showed a reduction in leaf area (by 18 percent) only in the 0.06 treatment. A reduction in shoot dry weight (by 21 percent) was apparent in barley at the 0.06 ratio but reductions were not at all evident in wheat. The second trial (Table 2.4, B) of three Ca/Na + Mg) ratios (1.25, 0.14 and 0.06) produced similar results. Barley leaf area was reduced 65 percent at the 0.06 Ca/(Na + Mg) ratio but wheat leaf area was unaffected. Barley dry weight was reduced 37 percent at the same ratio, but wheat again showed no effect.

Table 2.4 Effect of low Na, Mg and Ca concentrations on leaf area and shoot dry weight per plant of wheat and barley. Values are the means of 9 plants. Experiment 3.

Treatment				Wheat[a]		Barley[a]	
	Na	Mg	Ca	Leaf Area	Shoot Dry Weight	Leaf Area	Shoot Dry Weight
		(mM)		(cm^2)	(g)	(cm^2)	(g)
A.[b]							
	<0.1	2	3.0	609a	3.20a	626a	4.17a
	<0.1	5	2.0	612a	3.16a	652a	4.28a
	<0.1	5	1.0	599a	3.12a	558b	4.09a
	1.2	5	0.4	502b	2.86a	474c	3.30b
B.							
	<0.1	2	3.0	228a	0.65a	387a	1.03a
	0.5	5	0.75	220a	0.72a	340a	0.93a
	1.0	5	0.35	199a	0.68a	135b	0.65b

[a] Mean separation within columns for each experiment by Duncan's multiple range test, 5% level.

[b] Experiment A terminated after 36d rather than 21d.

Decreases in the Ca/(Na + Mg) ratio also increased the number of Ca-deficient leaves. In the first trial, a Ca/(Na + Mg) ratio of 0.2 produced 0.4 ± 0.2 Ca-deficient leaves per plant in wheat and 3.0 ± 0.7 in barley; a ratio of 0.06 resulted in 0.8 ± 0.2 and 8.9 ± 1.0 deficient leaves in wheat and barley, respectively (means of 9 plants ± s.e.). Deficient leaves were not apparent at the two higher ratios. A Ca/(Na + Mg) ratio of 0.14 in the second trial produced 0.8 ± 0.3 and 1.6 ± 0.4 Ca-deficient leaves in wheat and barley respectively; a ratio of 0.06 resulted in 8.6 ± 1.3 and 15.2 ± 1.2 Ca-deficient wheat and barley leaves, respectively (means of 9 plants ± s.e.). Again, no deficiencies were evident at the highest Ca/(Na + Mg) ratio.

Night-time humidity had no effect on the leaf area of wheat grown at low salinity, but did influence salinized plants (Experiment 4, Table 2.5). The salt x humidity interaction was significant at 0.6% and 1.6% probability levels in trials A and B, respectively. High humidity improved the growth of salinized plants (8.5 mM Ca, Ca/(Na + Mg) = 0.09) and reduced the number of Ca-deficient leaves. Calcium amendment (19 mM Ca, Ca/(Na + Mg) = 0.19) improved leaf area (by 41 and 66 percent in trials A and B, respectively) and reduced the number of Ca-deficient leaves at low humidity compared to non-amended plants. No significant effect of Ca amendment on leaf area was observed at high humidity, but the number of Ca-deficient leaves was reduced.

Humidity did not appear to influence the leaf Ca/(Na + Mg) ratios. At low humidity, the Ca/(Na + Mg) ratio was 1.33 ± 0.07, 0.16 ± 0.02 and 0.28 ± 0.03 in non-saline, saline and amended saline treatments, respectively (each value is the mean of two trials, each with nine plants, ± s.e.). Ratios at high humidity, in the same order, were 1.34 ± 0.01, 0.17 ± 0.01 and 0.29 ± 0.03.

Table 2.5 Effect of night-time humidity, Na and $MgSO_4$-salinity, and Ca on leaf area and number of Ca-deficient leaves of wheat. Values are the means of 9 plants. Experiment 4.

Treatment					Trial A[a,b]		Trial B[a]	
Night Humidity vpd (kPa)		Na (mM)	Mg (mM)	Ca (mM)	Leaf area (cm^2)	Ca-deficient leaves ($plant^{-1}$)	Leaf Area (cm^2)	Ca-deficient leaves ($plant^{-1}$)
0.8 - 1.0	Non-saline	<0.1	2	2.5	866a	0e	349a	0d
	Saline	50	50	8.5	419d	27a	151d	16.9a
	Amended saline	50	50	19.0	590c	10c	250b	2.4d
0.1 - 0.2	Non-saline	<0.1	2	2.5	904a	0e	352a	0d
	Saline	50	50	8.5	645bc	21b	205c	14.4b
	Amended saline	50	50	19.0	689b	5d	235bc	4.9c

[a] Mean separation within columns by Duncan's multiple range test, 5% level.
[b] Experiment terminated after 42d rather than 21d.

Leakage of UV-abosrbing substances from leaf tissue of wheat was influenced by salinity (Experiment 5, Table 2.6). Salinity increased RLR by 21%. Amendment of the saline solution with Ca resulted in no significant change in RLR compared to non-saline plants.

Table 2.6 Effect of saline solutions on the relative leakage ratio (RLR) of wheat-leaves. Experiment 5.

Treatment	Solution			RLR[a,b]
	Na	Mg	Ca	
	(m*M*)			
Non-saline	<0.1	2	2.5	0.0328b
Saline	50	50	10	0.0397a
Amended saline	50	50	20	0.0294b

[a] Values are means of the third and fourth leaves to emerge, each replicated 30 times (ie. 30 plants) in two experiments (n=60).

[b] Mean separation by Duncan's multiple range test, 5% level.

2.4 Discussion

Barley growth was not reduced as extensively as that of wheat in the presence of salt, supporting the view that barley is the more salt tolerant species (Maas and Hoffman, 1977). However, the Ca concentration of the salt solution had a marked effect on the relative salt tolerance of the two species, improving the growth of wheat but not barley. Hence, the difference in growth between the two species under saline conditions was due, in part, to differences in calcium nutrition.

Calcium has previously been found to alleviate the effects of salinity in cereals, but the comparisons have been made against very low calcium levels (eg. 0.1 mM) which are not generally representative of saline soils (Kawasaki and Moritsugu, 1978; Muhammed et al., 1987). It has been suggested that crop response to specific ions in artificially salinized media is often over estimated because of the use of these unusually low Ca levels (Maas and Grieve, 1987). Even so, Ca concentrations in some saline soils, although quite high, are insufficient to prevent reductions in tissue Ca content (Lynch and Lauchli, 1985; Janzen and Chang, 1987). In the present study, Ca deficiency symptoms occurred in young, expanding leaves of both wheat and barley at 3, 10, and even 18 mM Ca. Salinity also reduced tissue Ca levels in both species.

Reductions in growth and yield in a saline environment are the result of cumulative physiological changes arising from the osmotic and specific ion effects of salinity. Osmotic effects on Ca uptake due to drought or salinity have not been reported, but interactions with other minerals are known to be important. Ca/Na and Ca/Mg concentration (or activity) ratios have been related to growth rate under saline conditions (Madhok and Walker, 1969; Kent and Lauchli, 1985; Cramer and Lauchli, 1986; Cramer et al., 1986; Maas and Grieve, 1987; Muhammed et al., 1987; Grieve and Maas, 1988). Ca/cation ratios appear to be particularly relevant in serpentine or solonetzic soils (Proctor, 1970; Proctor et al., 1981; Carter et al., 1977, 1979) and in sulfate-dominated saline soils (Janzen and Chang, 1987). Recently, it has been shown that the high ionic strength which results from low Ca/cation ratios in saline solutions displaces Ca from the plasmalemma of root cells (Cramer et al., 1985; Lynch et al., 1987; Lynch and Lauchli, 1988), possibly contributing to salinity-induced Ca deficiencies.

Calcium/cation ratios are often achieved experimentally by progressively decreasing the salt concentration as the Ca concentration is increased, thereby maintaining a more consistent osmotic potential in the treatment solution. This may result, however, in an overestimation of the response to an increase in the Ca/cation ratio because of the concurrent reduction in Na or other saline cations. Despite a small reduction in osmotic potential, an increase in Ca concentration at constant Na plus Mg, or Mg alone, clearly promoted growth of wheat. This indicates that Ca was indeed limiting growth in the saline treatment.

Changes in the cation ratios of the treatment solutions were generally reflected in the cation ratios of leaf tissue. The Ca/(Na + Mg) ratios of both species decreased under saline conditions, but increased significantly with additional Ca only in wheat. Wheat consistently had higher Ca/(Na + Mg) ratios than barley in saline and amended saline treatments, even though growth under salinity was less than that of barley and showed a greater response to additional Ca. Wheat would appear to have higher Ca requirements than barley in the presence of salt, although this was not reflected in the leaf Ca relations of the two species.

The relative response of wheat and barley to Ca/(Na + Mg) ratio under non-saline conditions was the reverse of that found at high salinity. In this case, wheat showed fewer Ca-deficient leaves and less extensive growth reductions than barley. Cation antagonism under non-saline conditions is well known to influence tissue cation ratios (Mengel and Kirkby, 1987). Magnesium will depress Ca uptake (Ohno and Grunes, 1985), and low Ca/Mg ratios may lead to Ca deficiency and growth reductions (Key et al., 1962; Kawasaki and Moritsugu, 1979). Differences in Ca nutrition among species or genotypes, however, have rarely been characterized (Clarkson and Hanson, 1980), and to our knowledge have not previously been examined over broadly different salinities. In this case, the greater Ca requirement of wheat compared to barley appears to be unique to saline conditions, and is not related to a greater susceptibility of wheat to low Ca/(Na + Mg) and Ca/Mg ratios *per se*.

The substantially lower Ca/(Na + Mg) ratios (by 70-80 percent) of young, emergent leaves of wheat compared to older leaves suggests that the Ca nutrition of leaves in the crown is relatively poor, a situation often encountered in low or non-transpiring organs. Salinity appears to exacerbate the problem by reducing the Ca/(Na + Mg) ratio even further, inducing Ca deficiency symptoms in those leaves.

The modulation of the salt tolerance of wheat by Ca was influenced by humidity. High 24h humidity generally promotes growth and reduces transpiration and salt uptake rates, irrespective of salinity level (Hoffman and Jobes, 1978; Pitman, 1984; Lauter and Munns, 1987). In the case of wheat, a proportional increase in the growth of plants in both non-saline and saline conditions at high 24h humidity results in no change in salt tolerance (Hoffman and Jobes, 1978). In contrast, our results showed that high night-time humidity increased growth, but only in salinized plants, indicating an increase in salt tolerance under these conditions. The promotive effect of additional Ca on growth was not evident in the high humidity treatment. This suggests that an interaction between night-time humidity and Ca nutrition occurs under saline conditions, and is supported by the observation of reduced susceptibility of young leaves to Ca deficiency at high humidity.

Salinity-induced increases in leakage of UV-absorbing substances from wheat leaves were found to be ameliorated by additional Ca in the growing medium. Unlike previous measurements of salt-induced membrane leakiness, where low-salt leaf tissue has been incubated in saline solutions (Leopold and Willing, 1984), we have shown changes in leakiness based on _in vivo_ levels of salt and Ca. Calcium effects on leakage are not confined to saline situations, since increases in leakage were apparent also at low salinity when Ca/(Na + Mg) ratios were low (data not presented). It is conceivable that those changes in membrane leakiness related to Ca concentration or Ca/cation ratios may contribute to differences in growth.

There is an increasing awareness of the significance of Ca in salt tolerance. Differences in Ca nutrition at high salinity have recently been shown to occur among genotypes of barley (Lynch and Lauchli, 1985), rice (Grieve and Fujiyama, 1987) and sorghum (Grieve and Maas, 1988), and between salt-selected and unselected cells of alfalfa (Stavarek and Rains, 1984). In all cases, salt tolerance appears to be related to improved Ca concentrations or Ca/cation ratios in the tissue of tolerant plants. Based on our findings, differences between species do not seem to be related to differences in Ca concentration in the tissue, but rather to differences in Ca utilization or requirements. In this respect, salt-sensitive species may be more dependent on Ca availability than are salt tolerant species. Breeding or selection programs for salt tolerance must therefore take into account the critical role of Ca, with appropriate consideration given to the Ca relations of each species.

2.5 Summary

The effect of Ca on the salt tolerance of wheat and barley was examined in solution culture. Wheat showed a greater reduction in growth and a higher incidence of foliar Ca deficiency symptoms than barley when grown under $MgSO_4$ or Na_2SO_4 plus $MgSO_4$ salt stress. Amendment of the saline solution with Ca to increase the Ca/(Na + Mg) ratio ameliorated the effects of salt, but more so in wheat than in barley. At least part of the difference in salt tolerance between the two species must therefore relate to differences in Ca nutrition. The greater response of wheat to Ca was not due to a lower Ca status in leaf tissue; on the contrary, although Ca amendments improved tissue Ca/(Na + Mg) ratios in both species, salinized wheat had equivalent or higher Ca content, and higher Ca/(Na + Mg) ratios than did barley. The higher Ca requirement of wheat is apparently specific to a saline situation; at low salinity, wheat growth was not reduced as extensively as that of barley as Ca/(Na + Mg) ratio was decreased. High night-time humidity dramatically improved wheat growth under saline conditions, and compensated for low solution Ca concentrations. Increasing the Ca concentration of the saline solution had no effect on growth in the high humidity treatment. Membrane leakage from leaf tissue of wheat grown under saline conditions was increased compared to tissue from non-saline plants. Plants grown in Ca-amended saline solutions showed no increase in membrane leakage. These results demonstrate the importance of Ca interaction with salinity stress, and indicate differences in species response.

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3.0 INFLUENCE OF CALCIUM ON THE COMPARATIVE SALT TOLERANCE OF COMMERCIAL AND WILD BARLEY SPECIES.

3.1 Introduction

Foxtail barley (*Hordeum jubatum*), is a wild species that can inhabit saline environments (Best et al., 1978) at soil pH values exceeding 8.0, electrolyte conductivities of 1.6 S/m and concentrations of Na, Mg, and SO_4 in soil saturation extracts in the range from 100-200 mol m^{-3} (Dodd, 1963). Saline and saline-sodic soils characteristic of the Canadian prairie provinces generally contain high concentrations of magnesium sulfate and sodium sulfate salts (Redmann and Fedec, 1987). Saline soils adversely affect plant growth and development and reduce crop productivity although barley represents one of the most salt tolerance crop species (Maas, 1986). Soil salinity is a major factor influencing the growth and yield of crop plants of importance to agriculture in Saskatchewan (Fowler and Hamm, 1980). There is a need to develop barley varieties that are adapted to local salinity conditions which yield grain of high quality and quantity. If the salt tolerance of *H. jubatum* can be demonstrated to be greater than varieties of *H. vulgare* then *H. jubatum* germplasm may be a useful source of salt tolerance genes which could be transferred to commercial lines through interspecific crosses or by molecular genetic techniques.

Calcium availability under conditions of salt stress has been shown to be an important factor in mitigating the adverse affects of salinity on a number grass species. Salt tolerance and sodium-induced calcium deficiency of corn (*Zea mays* L.) (Maas and Grieve, 1987), sorghum (*Sorghum bicolor* (L.) Moench) (Grieve and Maas, 1988), and rice (*Oryza sativa* L.) (Grieve and Fujiyama, 1987) have been shown to be strongly influenced by the Na/Ca ratios of the salinized growth medium. Soil salinity reduces the calcium content of barley foliage and nutrient solution applied NaCl inhibits the root to shoot transport of calcium (Lynch and Lauchli, 1985). The adverse affects of $MgSO_4$ and $NaSO_4$ based soil salinity have been shown to reduce barley yield when Ca/total cation ratios fell below 0.15 (Carter et al., 1979) or when expressed in terms of ion activity, at $a_{Ca}/a_{Total\ Cations}$ below 0.09 (Janzen and Chang, 1987).

The objectives of this study were to determine whether differences in salt tolerance between *H. vulgare* and *H. jubatum* actually do exist, to quantitate differences in growth and ion accumulation between *H. vulgare* cv. Harrington, cv. Bonanza and *H. jubatum* in response to salinity, and to examine the effects of Ca on the relative salt tolerance of these species.

3.2 Methods

Foxtail barley was collected from a site on the Univ. of Saskatchewan campus. Analysis of the saturation extract from soil collected at this site provided the following data: the soil pH was 7.4, the electrolyte conductivity (EC) was 0.08 $S\ m^{-1}$, and the sodium absorption ratio (SAR) was 0.4. Harrington and Bonanza seed was obtained from the Crop Development Center, Univ. of Saskatchewan.

Harrington and Bonanza seeds were germinated at room temperature on moist filter paper; foxtail barley seeds required a cold pretreatment prior to germination. Following germination the 2-day old seedlings were placed onto floating platforms and grown in plastic 5 L tubs containing aerated nutrient solution. Salt stress treatments were initiated for the commercial lines at this point, however to compensate for slow growth of foxtail barley seedlings, salt treatments for these seedlings were delayed for 14 d.

The nutrient solution had the following composition with concentrations given in mol m^{-3}: 0.2 $NH_4H_2PO_4$, 2.0 $NaNO_3$, 2.5 $CaCl_2$, 0.75 K_2SO_4, 1.8 $MgSO_4$, 0.007 Fe (sodium ferric ethylenediamine-bis-2-hydroxyphenol acetate), 0.025 H_3BO_4, 0.002 $MnSO_4$, 0.002 $ZnSO_4$, 0.0005 $CuSO_4$, 0.0005 Na_2MoO_4, and 0.026 Na_2SiO_3 (used to maintain stable pH). The initial pH of the nutrient solution was 6.8 and ranged from 6.8 - 7.2 during the course of the experiment.

Plants were salinized by the addition of salts to nutrient solutions at a rate not exceeding -0.178 MPa d^{-1}. Increasing concentrations of $MgSO_4$, Na_2SO_4, and $CaCl_2$ were added at fixed salt ratios, Mg:Na:Ca=20:20:1 and Mg:Na:Ca=5:5:1, that provided a range of treatments with increasing EC at two calcium:total cation ratios (Ca/TC), 0.02 and 0.07, respectively. The Ca/TC of control solutions was 0.32 with a corresponding Mg:Na:Ca ratio of 0.72:0.80:1. The EC, SAR and osmotic potential values for the salt treatments are given in Table 3.1. Sodium absorption ratios were calculated from the equation (Richards, 1954):

$$SAR = Na/ \sqrt{(Ca+Mg)/2}$$

Plants were grown in a Conviron growth chamber at 21/18 °C

Table 3.1 *Electrolyte conductivity, sodium absorption ratio, and osmotic potential*[†] *values for salt treatments.*

	Mg:Na:Ca=5:5:1			Mg:Na:Ca=20:20:1		
Treatment	**EC (S/m)**	**SAR**	**OP (bar)**	**EC (S/m)**	**SAR**	**OP (bar)**
control[‡]	0.12	1.0	-----	0.12	1.0	-----
2	0.29	4.1	-1.0	0.26	4.4	-0.9
3	0.48	5.8	-2.0	0.45	6.2	-1.8
4	0.65	7.1	-3.0	0.61	7.6	-2.7
5	0.81	8.2	-4.0	0.75	8.7	-3.6
6	0.96	9.1	-5.0	0.90	9.8	-4.5
7	1.13	10.0	-6.0	1.04	10.7	-5.3
9	1.38	11.5	-7.6	1.30	12.3	-7.1
11	----	----	----	1.66	13.8	-8.9
13	----	----	----	1.88	15.1	-10.7

[†] calculated values

[‡] control solution Mg:Na:Ca=0.7:0.8:1

day/night temperatures under a 16/8 h light/dark period. The average light intensity in the chamber was 530 umol photon m^{-2} s^{-1}. Plants were harvested 21 d after the onset of salt stress. Leaf areas were measured with a leaf area meter (LI-COR LI-3000, LI-COR, Lincoln, NE). Plant dry weights were determined on oven dried material. Plant tissues were digested with a nitric-perchloric-hydrochloric acid digest and ions determined by plasma emission spectrophotometry (Applied Research Laboratories, Model 3410 ICP). The ion selectivity ratios for K:Na selectivity ($S_{K,Na}$) were determined from the equation (Pitman, 1976):

$$S_{K,Na} = (K\ content/[K]\ medium)/(Na\ content/[Na]\ medium)$$

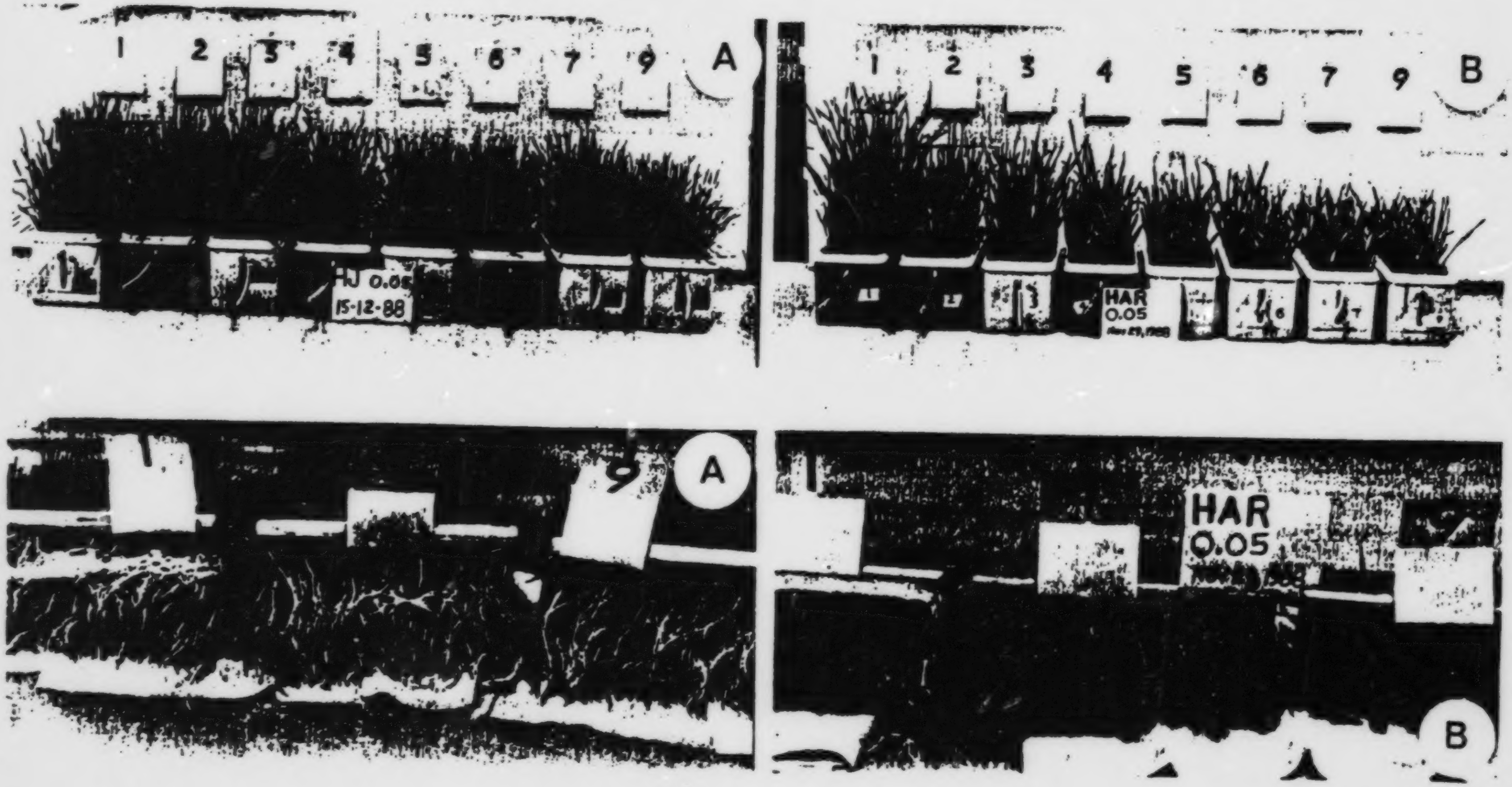
Leaf area data is the mean of 36 randomly selected plants, 12 from each experiment. Dry weight and ion analysis data is the mean of one determination taken from each of the three experiments.

3.3 Results

3.3.1 Salinity effects on plant growth

Salt stress more adversely affected the growth of *Hordeum vulgare* cv. Harrington and cv. Bonanza than foxtail barley (*Hordeum jubatum*) in comparative solution culture experiments when seedlings were stressed for 21 days. Salts supplied to nutrient solutions in the form of increased concentrations of $MgSO_4$, Na_2SO_4, and $CaCl_2$ induced visual symptoms of ion stress in Harrington and Bonanza plants (Fig. 3.1.0, 3.1.1, 3.2); the symptoms being more pronounced when salt stress was applied at the low Ca ratio (Mg:Na:Ca=20:20:1) as opposed to the high Ca ratio (Mg:Na:Ca=5:5:1).

Stress related growth reductions were quantified by leaf area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HAR
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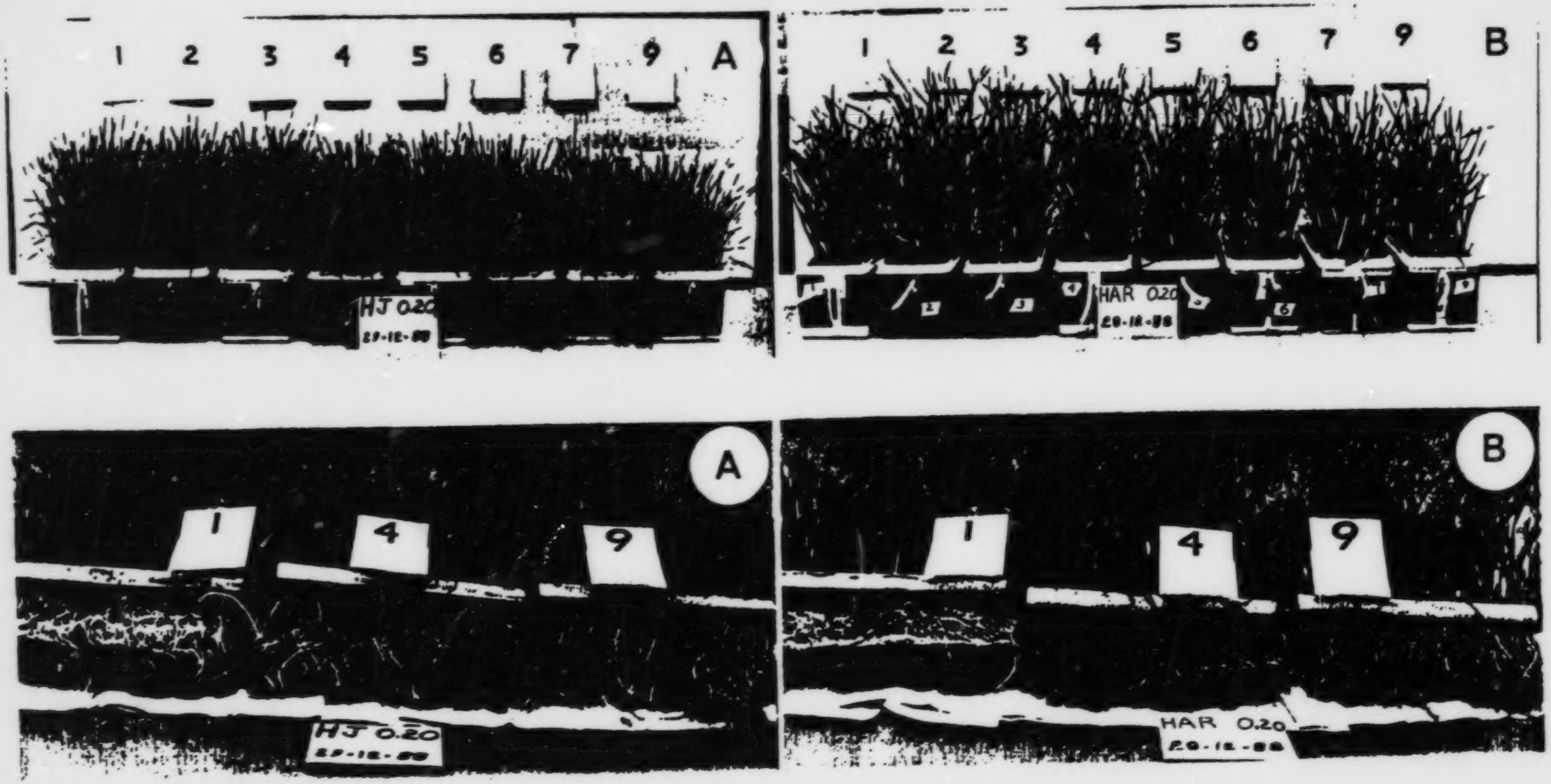
Fig. 3.1.0 *H. jubatum* (A), and Harrington (B) plants subjected to increasing salinity at a Ca/TC of 0.02, where the ratio of salts in the stress solution was Mg:Na:Ca=20:20:1. For control plants (1) the solution Mg:Na:Ca=0.7:0.8:1, with an EC of 0.12 S/m. See Table 3.1 for EC, SAR, and OP values for the salt treatments (2-9).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HAR 0.20

Fig. 3.1.1 *H. jubatum* (A), and Harrington (B) plants subjected to increasing salinity at a Ca/TC of 0.07, where the ratio of salts in the stress solution was Mg:Na:Ca=5:5:1. For control plants (1) the solution Mg:Na:Ca=0.7:0.8:1, with an EC of 0.12 S/m. See Table 3.1 for EC, SAR, and OP values for the salt treatments (2-9).

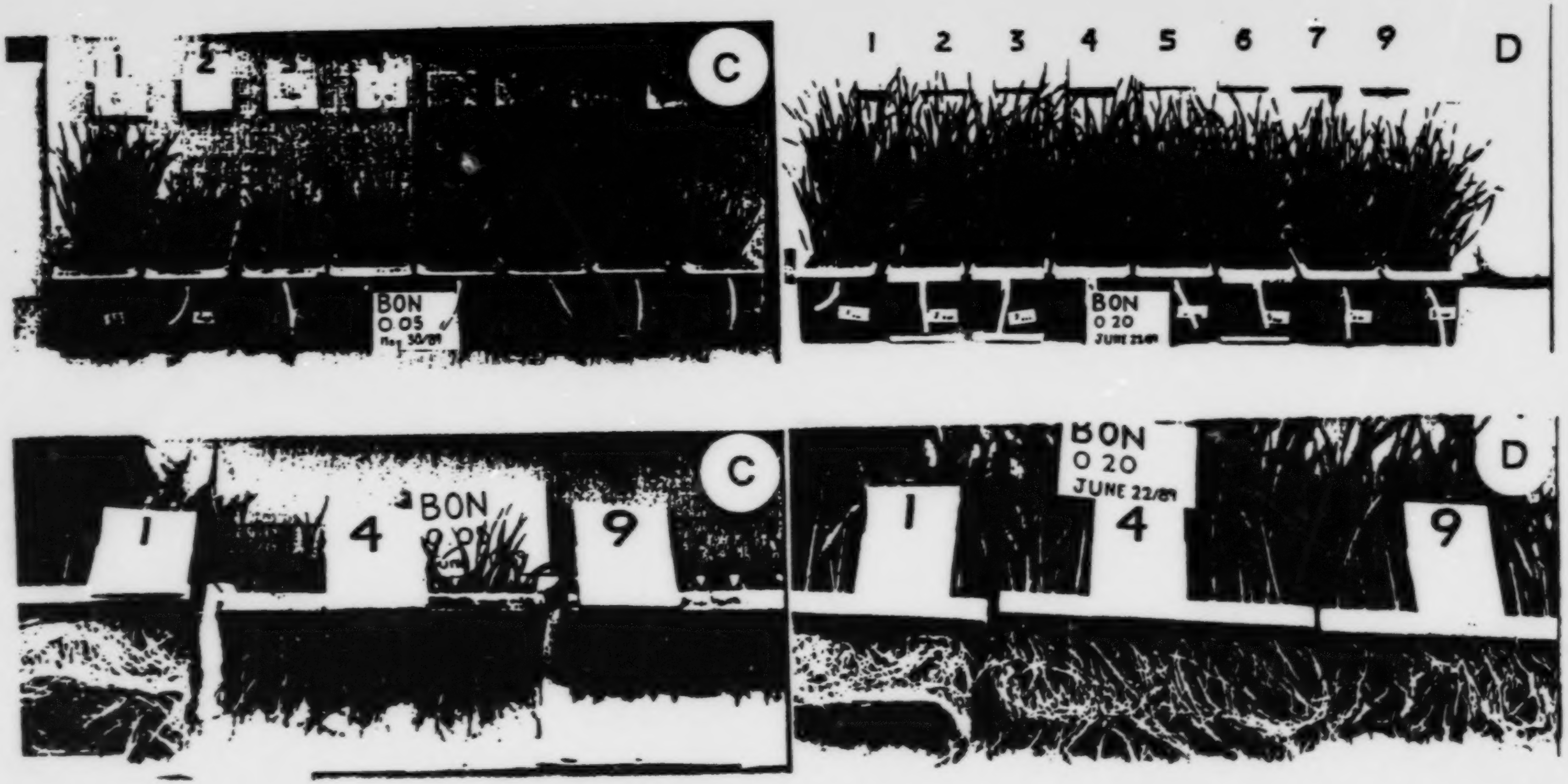
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Fig. 3.2 Bonanza plants subjected to increasing salinity at two Ca/TC ratios: 0.02 (C), and 0.07 (D). For plants shown in (C) the ratio of salts in the stress solution was Mg:Na:Ca=20:20:1, and for those in (D) the Mg:Na:Ca=5:5:1. For control plants (1) the solution Mg:Na:Ca=0.7:0.8:1, with an EC of 0.12 S/m. See Table 3.1 for EC, SAR, and OP values for salt treatments (2-9).

and dry matter measurements. Decreases in leaf area were more strongly influenced by the Ca/TC than by the EC of the salt solutions (Fig. 3.3). In *H. jubatum* plants leaf area was independent of Ca/TC but decreased slightly in response to increasing EC (Fig. 3.3). Salt stress at the higher Ca/TC provided leaf areas for Harrington and Bonanza plants that were equivalent to foxtail barley plants.

Dry matter data indicate that the primary adverse effect of salinity stress was upon the root growth of the commercial lines and that production of root dry matter was significantly modulated by the Ca/TC of the saline solution. Root growth of Harrington and Bonanza plants was more severely affected than foliage growth at every salt treatment. Low Ca/TC salt stress conditions produced severely stunted roots (Fig. 3.1.0, 3.2), substantial reductions in root dry weight (Fig. 3.4, 3.5) and a strong reddish-brown discoloration of root tissue as compared to control roots. Salt treatments applied at the higher Ca/TC reduced root discoloration (Fig. 3.1.1, 3.2) and significantly increased root dry weight (Fig. 3.4, 3.5), though not to the levels of control plants. In contrast to the commercial lines, foxtail barley plants showed little if any visible symptoms of salinity stress in root or foliage tissue (Fig. 3.1.0, 3.1.1). Unlike Harrington and Bonanza, root dry weight of foxtail barley plants was only minimally affected at the lower Ca/TC salt stress and at the higher Ca/TC treatments root dry weights exceeded or were equivalent to control plants (Fig. 3.4).

Visual symptoms of salt stress evident in Harrington and Bonanza foliage included stunted growth, chlorosis, and signs of

Ca deficiency in the immature leaves. Salinity-induced foliar injury in the commercial lines was alleviated when these plants were stressed at the higher Ca/TC treatments and shoot dry matter production was essentially equivalent to control plants (Fig. 3.4, 3.5). Foxtail barley plants showed similar increases in dry matter production at the various salt treatments when salt stressed at the higher Ca/TC.

Dry weight data reformulated to show the root:shoot ratio (R/S) for these three species in relation to salt stress is shown in Fig. 3.6. The R/S for Harrington and Bonanza under low Ca/TC conditions declined significantly with increased salinity, reaching 25% and 21% of control values, respectively at 1.30 S/m. Salt stress conditions that employed the higher Ca/TC substantially improved both Harrington and Bonanza R/S across the range of salt treatments having values of 65% and 61% of control, respectively at 1.38 S/m. In contrast, the R/S in foxtail barley was only affected under low Ca/TC stress conditions; dropping to 73% of control values at 1.30 S/m. Salt stress at the higher Ca/TC did not significantly reduce the R/S of the wild species which declined to 91% of control plants at 1.38 S/m.

3.3.2 Tissue ion composition

Ion analysis data revealed significant quantitative differences in ion partitioning between root and shoot tissue of *H. jubatum*, Harrington and Bonanza barley. The concentrations of Mg, Na, Ca and SO_4 increased in plant tissues in response to increased concentrations of these ions in the solution cultures.

Clear differences in Na accumulation were evident among the species with *H. jubatum* being the most efficient Na excluder from root and notably shoot tissue (Fig. 3.7.1, 3.7.2). Sodium levels in *H. jubatum* and Harrington shoots were unaffected by the Ca/TC, whereas in Bonanza shoots while the rate of Na accumulation remained unchanged at the higher Ca/TC the total concentration of Na in the tissue was reduced by an average of 32% across the range of salt treatments. In roots the higher Ca/TC reduced both the rate of accumulation and the total Na content of Harrington and Bonanza tissue. *H. jubatum* roots were the best Na excluders however at the higher Ca/TC salt stress, while Bonanza roots displayed Na exclusion properties similar to *H. jubatum*.

Potassium distributions in control root and shoot tissues of all three species showed a preferential accumulation of K in the aerial potions of the plant with characteristic by shoot:root ratios for K 1.36, 2.36 and 1.94 for foxtail, Harrington and Bonanza barley, respectively (Fig. 3.8.1, 3.8.2). Harrington shoots had the highest K content at the lowest salt treatment compared with foxtail and Bonanza barley which were equivalent. Neither commercial line could maintain initial K levels, which decreased with increasing salt stress. However, stress at the higher Ca/TC reduced both the rate and total amount of K decline in shoot tissue. *H. jubatum* maintained constant levels of K in shoots and the K content was independent of the Ca/TC of the salt solutions. Under control conditions, roots of foxtail barley plants accumulated 46% and 57% more K than Harrington and Bonanza roots, respectively (Fig. 3.8.1, 3.8.2). However in response to salinity each species showed differing trends when stressed under the two Ca conditions. At the higher Ca/TC salt stress, *H. jubatum* roots accumulated less total K but the amount of K accumulated increased with increasing EC. Harrington roots by contrast showed both an increased rate of K accumulation as well as a real increase in K content across the range of salt treatments at the higher Ca/TC stress. Bonanza roots showed a response that was intermediate between *H. jubatum* and Harrington. Salt stress at the lower Ca/TC, while having intially greater levels of K compared to the higher Ca ratio, declined with increased EC; whereas under the high Ca/TC salinity stress the K concentration increased with increasing EC.

Calcium partitioning between roots and shoots of control plants displayed a strong preference for transport and accumulation of Ca into foliage tissue this being reflected by shoot:root Ca ratios of 2.0, 5.4 and 3.8 for foxtail barley, Harrington and Bonanza, respectively (Fig. 3.9.1, 3.9.2). When the commercial lines were salinized at the higher Ca ratio (Mg:Na:Ca=5:5:1) the levels of shoot Ca in Harrington and Bonanza plants were 3.0- and 2.2-fold greater, respectively across the range of salt treatments than plants salinized at the lower Ca ratio (Mg:Na:Ca=20:20:1). Under identical conditions, *H. jubatum* shoot Ca levels exceeded both commercial lines by approximately 59%. Root Ca levels in control plants were 1.9- and 2.2-fold greater in *H. jubatum* than Harrington and Bonanza, respectively. The wild species maintained this advantage over the commercial lines in all salt treatments.

Magnesium concentrations increased in shoot and root tissues of all three species with elevated levels of Mg in the solution cultures (Fig. 3.10.1, 3.10.2). At the lower Ca/TC stress, Harrington and Bonanza shoot and root Mg levels were substantially greater than foxtail barley. Root Mg concentrations of Harrington and Bonanza were reduced to the level found in *H. jubatum* roots at the higher Ca/TC; however a reduction in foliar Mg levels was also observed in Bonanza plants.

Partitioning of S in plants salinizied at the low Ca ratio (Mg:Na:Ca=20:20:1) revealed an inverse relationship between the wild species and the commercial cultivars (Fig. 3.11.1, 3.11.2). We assumed that the levels of S determined in plant tissues by plasma emission spectroscopy are proportional to SO_4 levels in these tissues. Foxtail barley plants accumulated more S in root tissue than shoot tissue; the reverse held true for Harrington and Bonanza. Significant reductions in S were observed in Bonanza shoot tissue and *H. jubatum* root tissue under the high Ca ratio stress conditions (Mg:Na:Ca=5:5:1).

3.3.3 Ion selective uptake in roots and shoots

Foxtail barley was superior to Harrington in K:Na selectivity ($S_{K,Na}$) in root and foliage tissue (Fig. 3.12.1). The K:Na selectivity of the wild species was independent of the Ca/TC used in the stress solutions. Calcium had a positive modulating effect on the $S_{K,Na}$ values for Harrington roots but not shoots when the Ca/TC of the salt solutions was raised from 0.02 to 0.07. For Bonanza plants both shoot and root $S_{K,Na}$ values were positively modulated by this increase in Ca/TC of 0.07 (Fig. 3.12.2).

The comparative K/Na ratios for salinized *H. jubatum* and

Table 3.1 *Electrolyte conductivity, sodium absorption ratio, and osmotic potential[†]values for salt treatments.*

	Mg:Na:Ca=5:5:1			Mg:Na:Ca=20:20:1		
Treatment	EC (S/m)	SAR	OP (bar)	EC (S/m)	SAR	OP (bar)
control[‡]	0.12	1.0	-----	0.12	1.0	-----
2	0.29	4.1	-1.0	0.26	4.4	-0.9
3	0.48	5.8	-2.0	0.45	6.2	-1.8
4	0.65	7.1	-3.0	0.61	7.6	-2.7
5	0.81	8.2	-4.0	0.75	8.7	-3.6
6	0.96	9.1	-5.0	0.90	9.8	-4.5
7	1.13	10.0	-6.0	1.04	10.7	-5.3
9	1.38	11.5	-7.6	1.30	12.3	-7.1
11	----	----	----	1.66	13.8	-8.9
13	----	----	----	1.88	15.1	-10.7

[†] calculated values

[‡] control solution Mg:Na:Ca=0.7:0.8:1

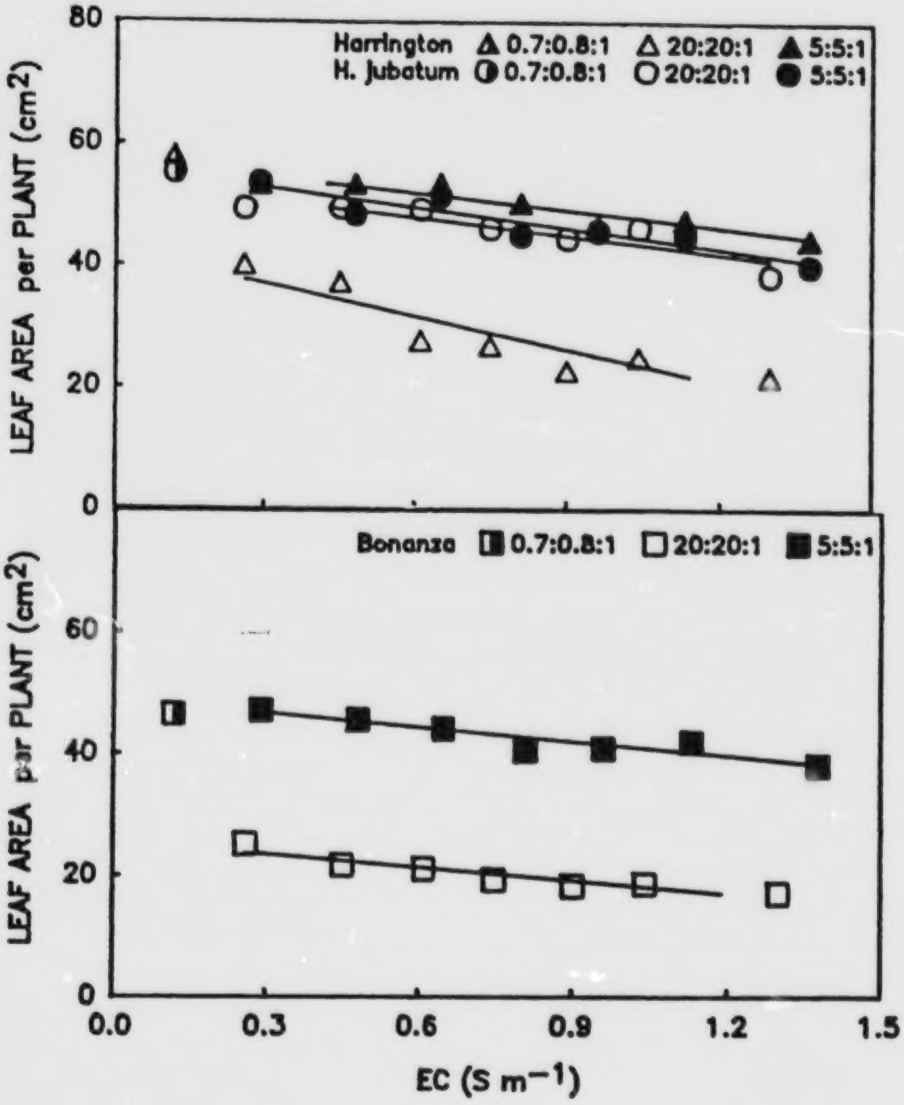

Fig. 3.3 Leaf area of *H. jubatum*, Harrington, and Bonanza as a function of Ca/TC and solution electrolyte conductivity (EC).

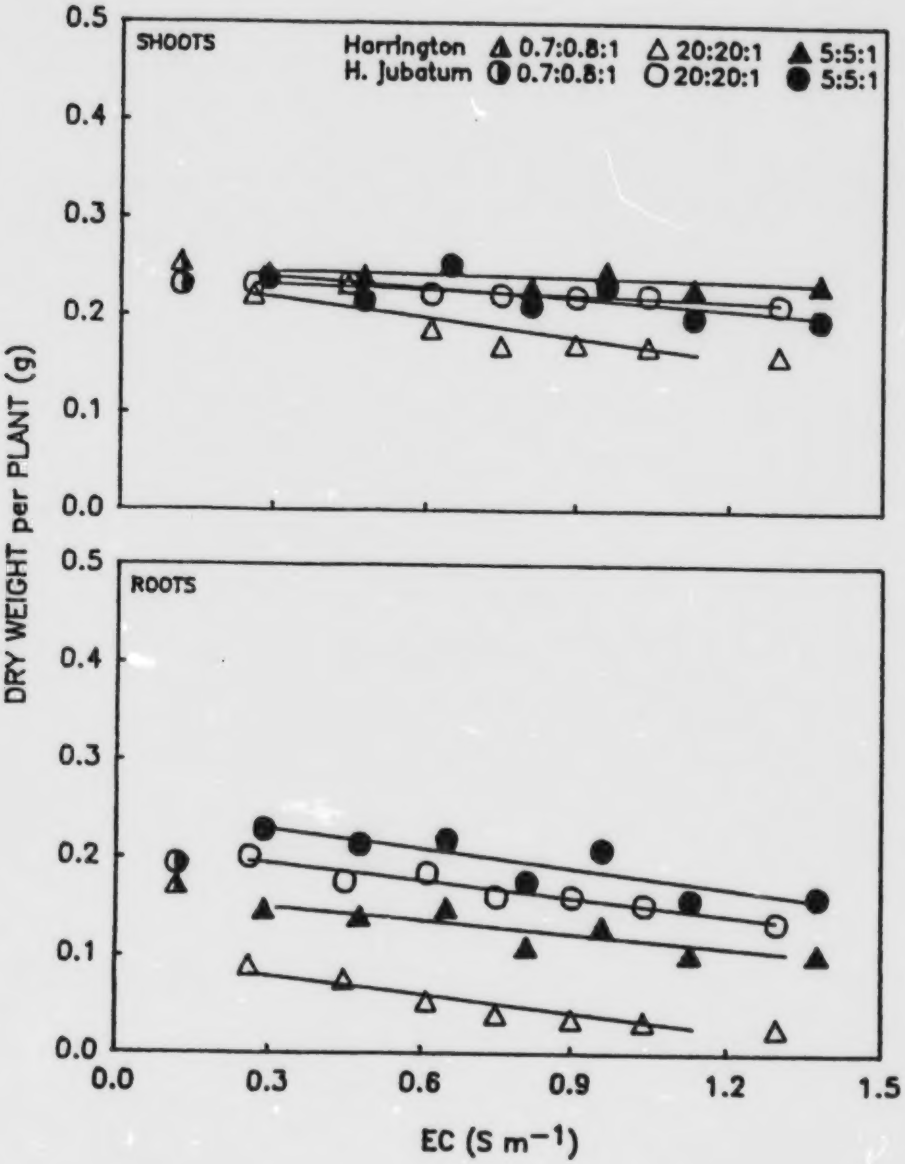

Fig. 3.4 Dry weight of *H. jubatum* and Harrington shoots and roots as a function of increasing salt stress applied at two Ca/TC ratios.

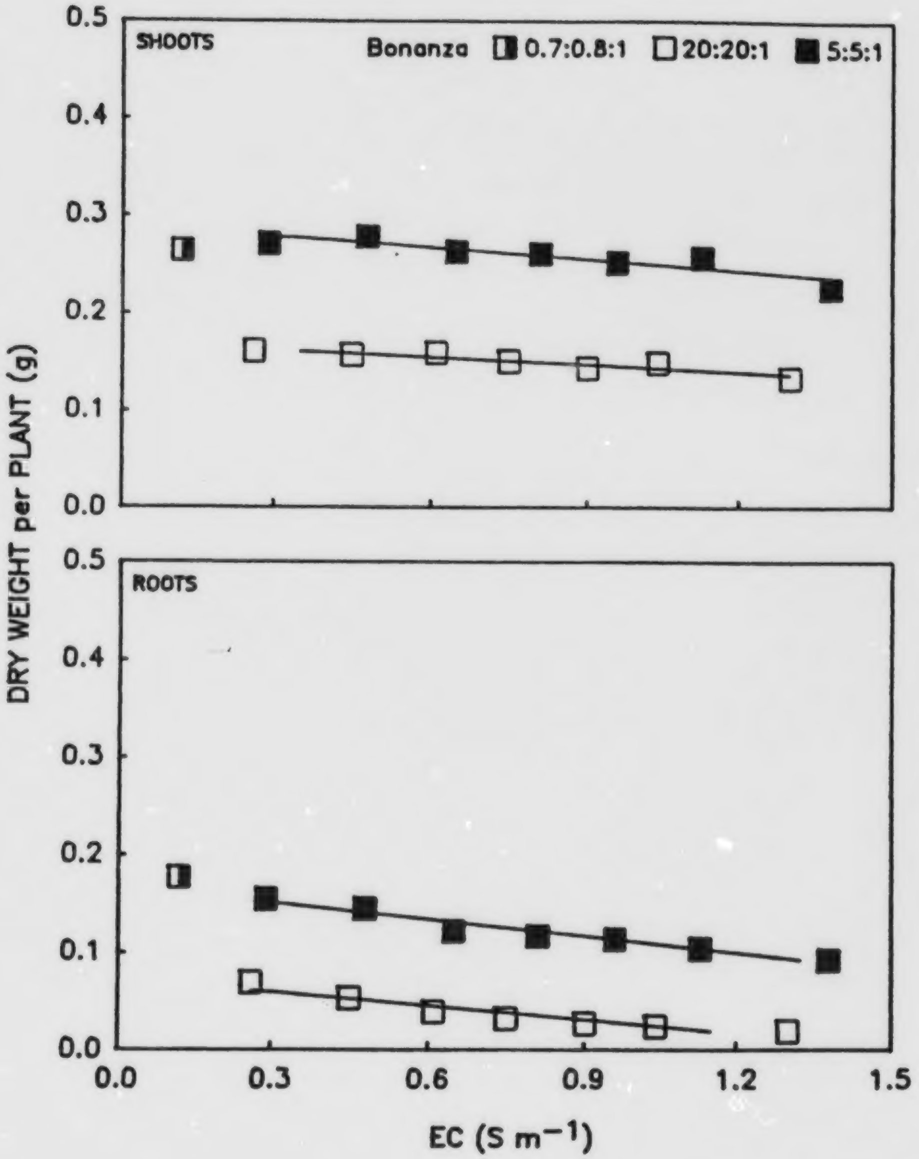

Fig. 3.5 Dry weight of Bonanza shoots and roots as a function of increasing salt stress applied at two Ca/TC ratios.

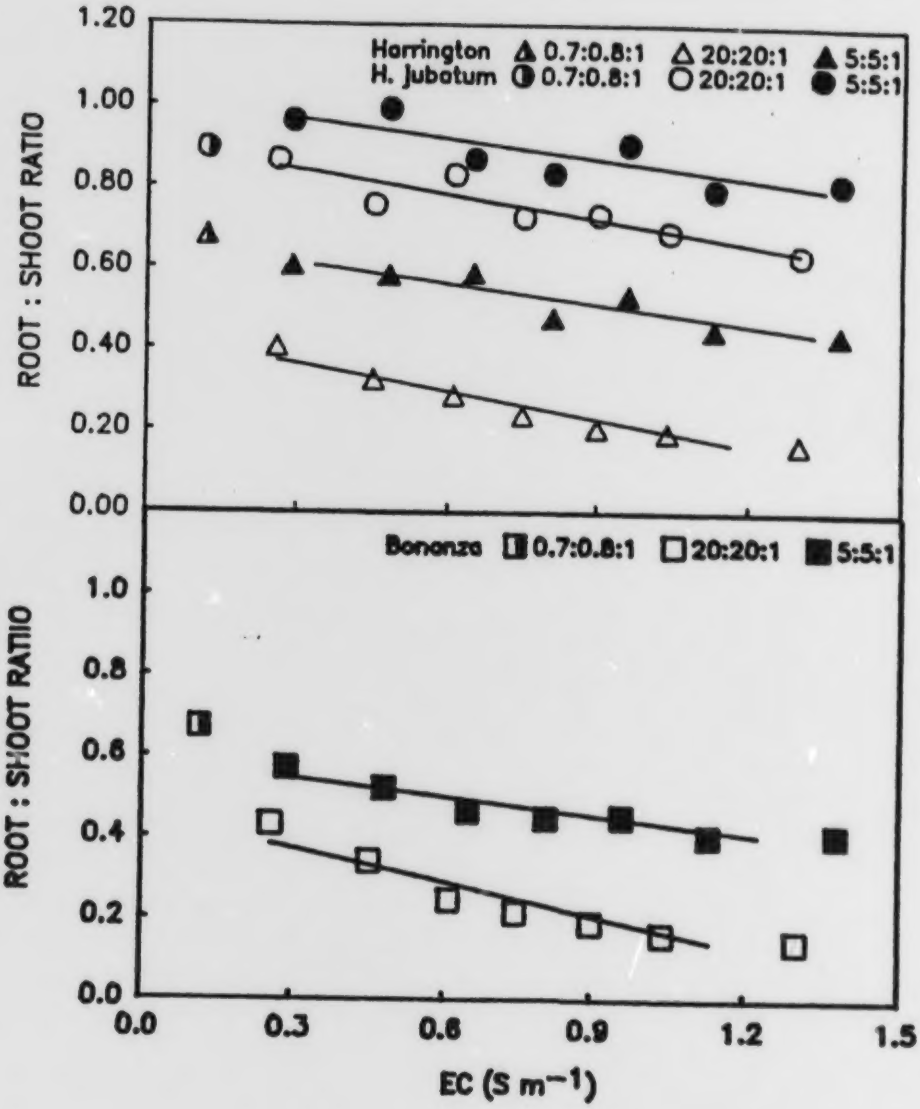

Fig. 3.6 Root:shoot ratios of *H. jubatum*, Harrington, and Bonanza plants as a function of EC and Ca/TC.

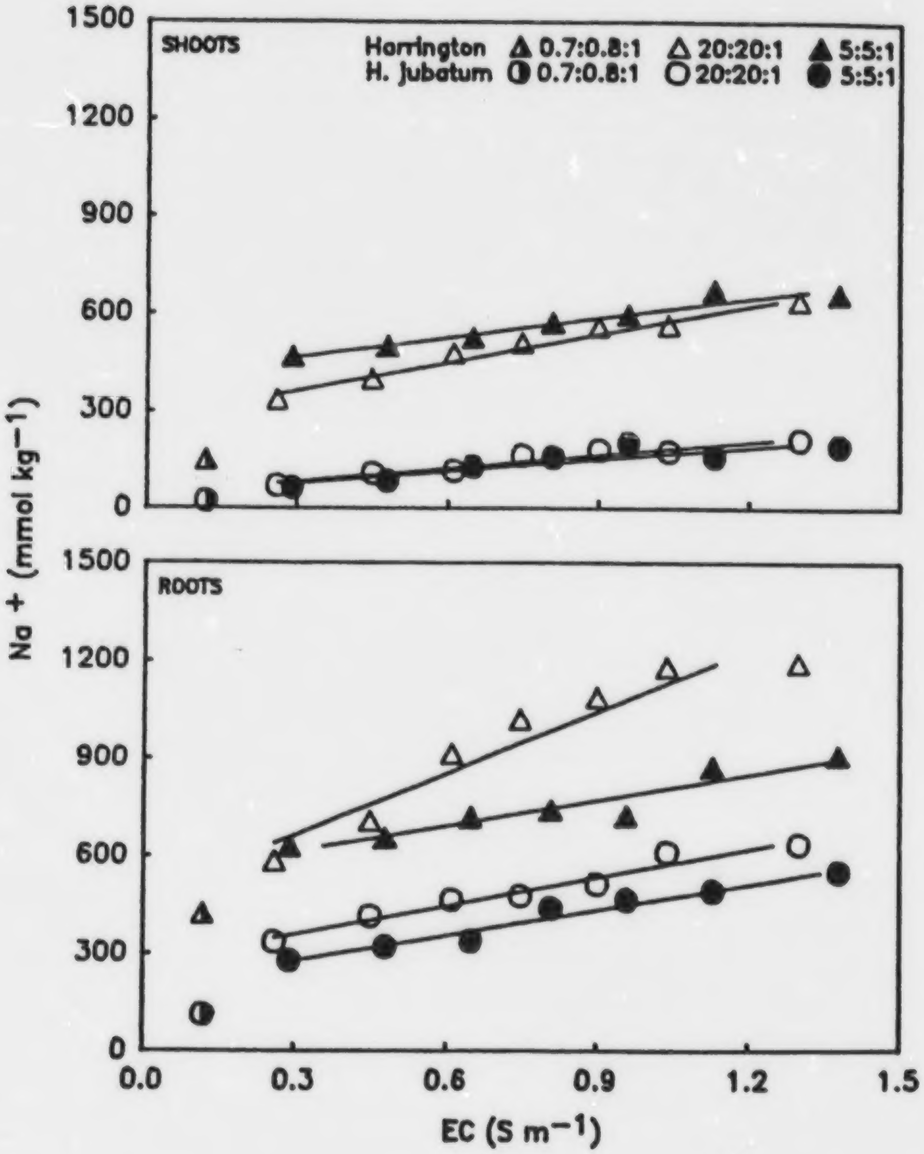

Fig. 3.7.1 Sodium content of *H. jubatum* and Harrington shoot and root tissue as a function of EC and Ca/TC.

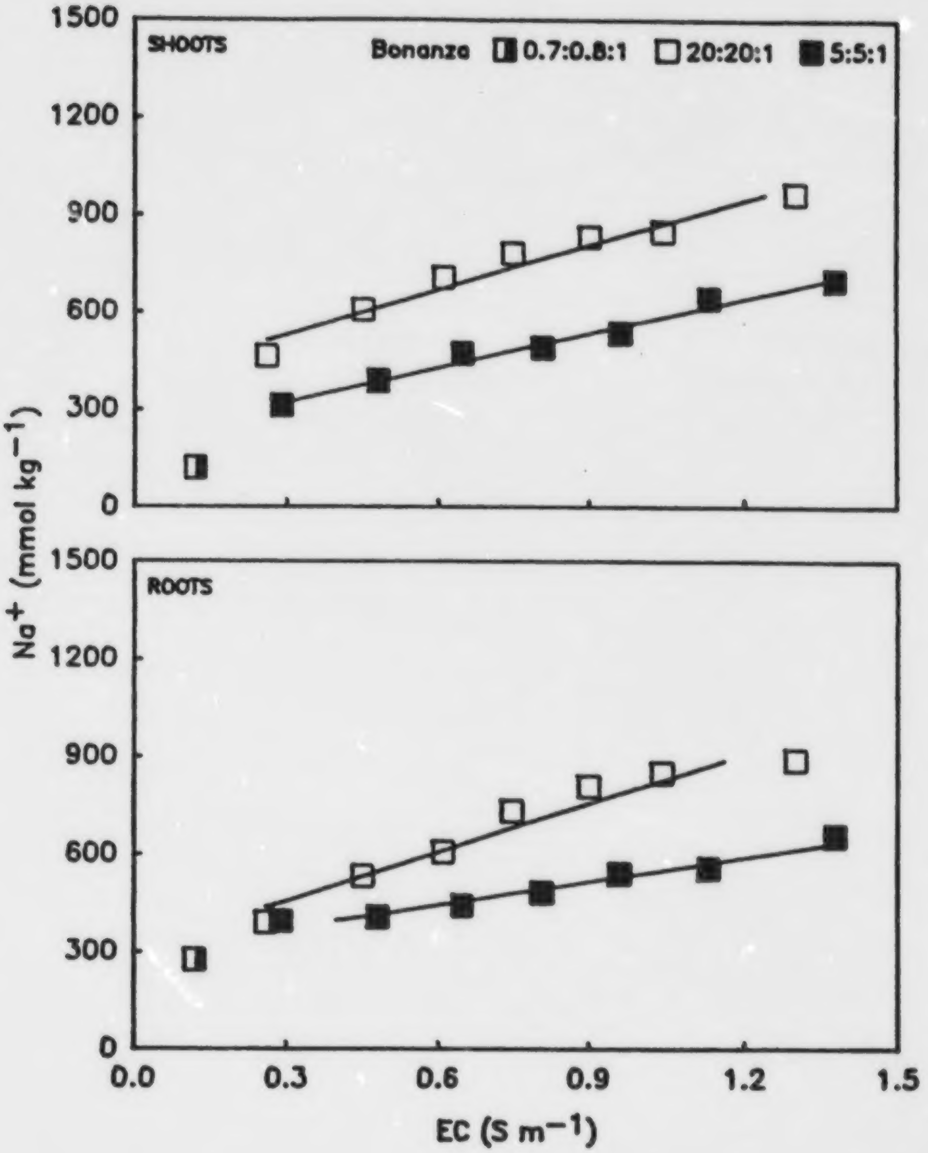

Fig. 3.7.2 Sodium content of Bonanza shoot and root tissue as a function of EC and Ca/TC.

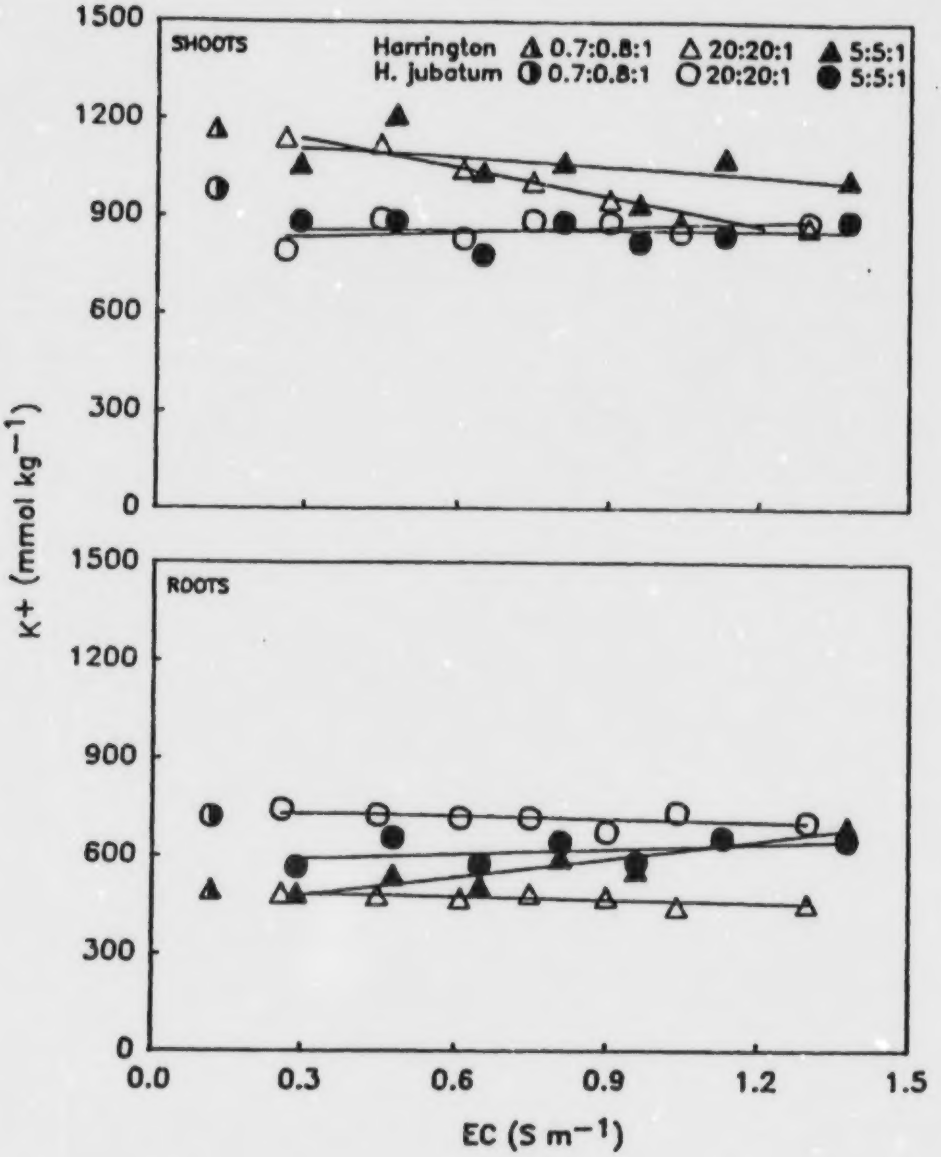

Fig. 3.8.1 Potassium content of *H. jubatum* and Harrington shoot and root tissue as a function of EC and Ca/TC.

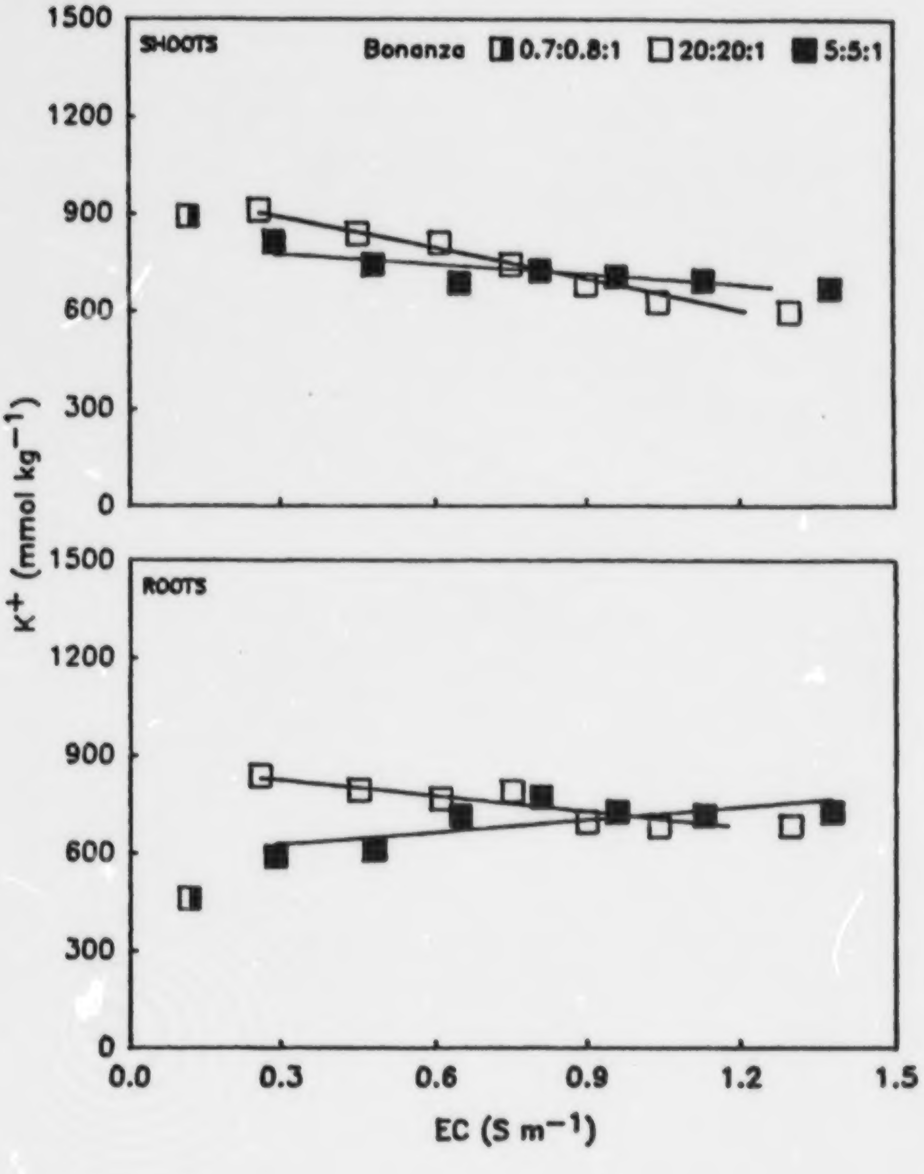

Fig. 3.8.2 Potassium content of Bonanza shoot and root tissue as a function of EC and Ca/TC.

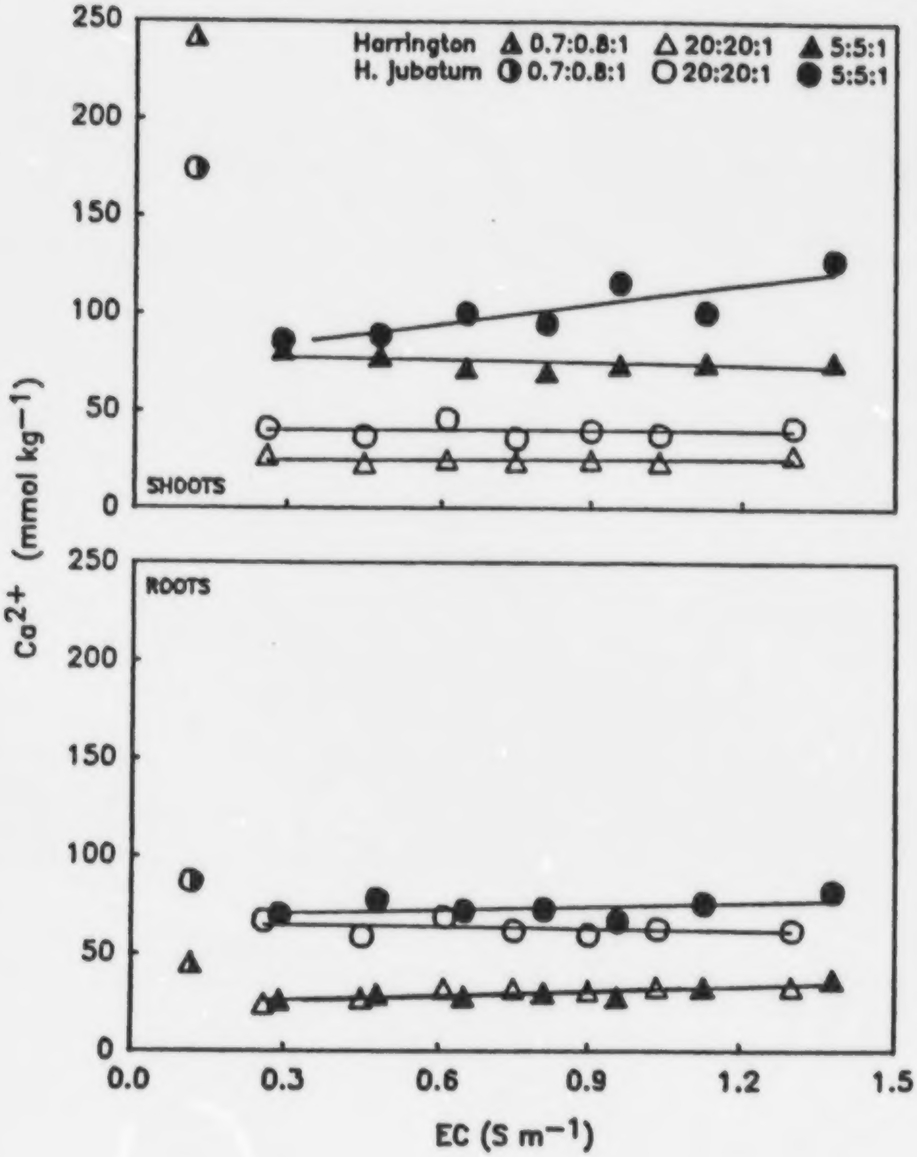

Fig. 3.9.1 Calcium content of *H. jubatum* and Harrington shoot and root tissue as a function of EC and Ca/TC.

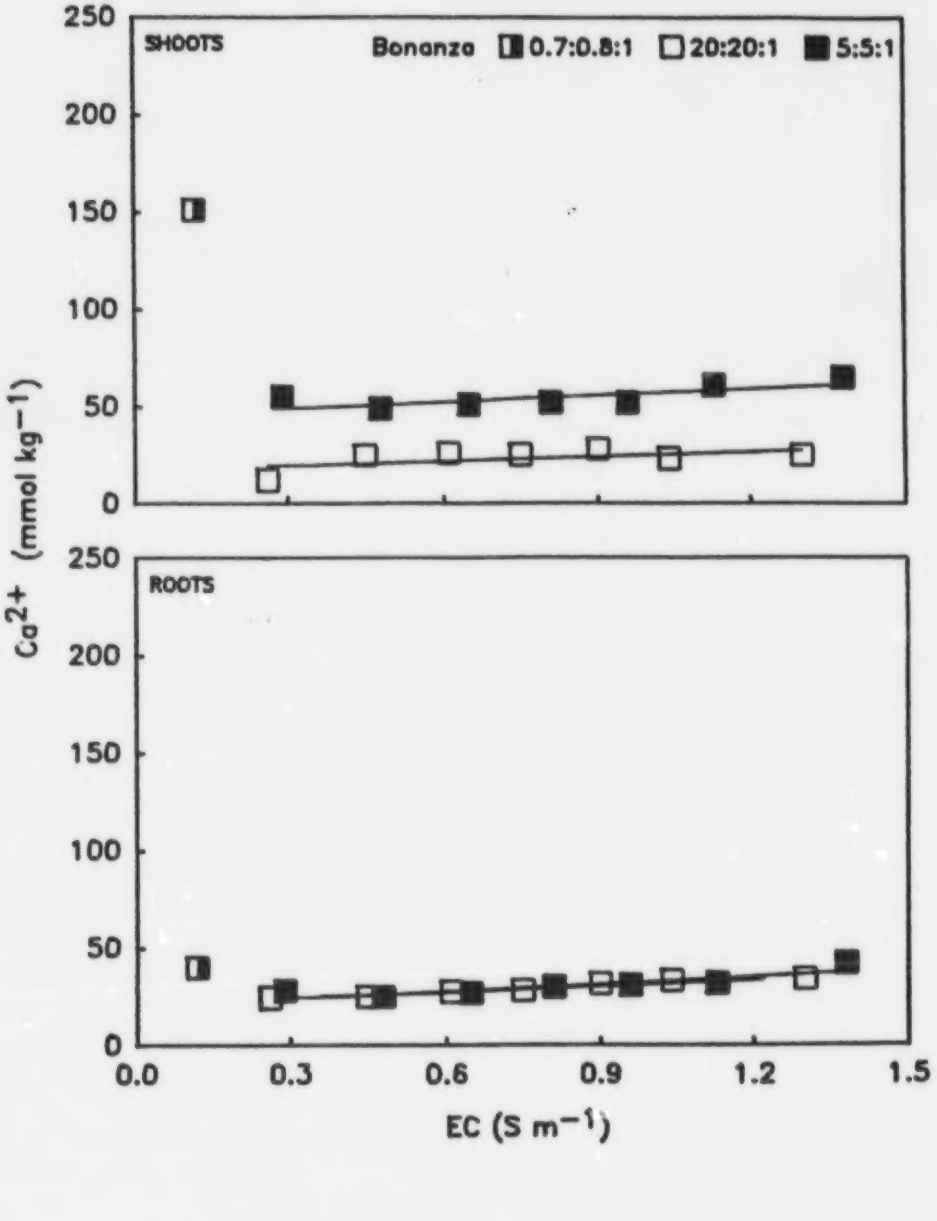

Fig. 3.9.2 Calcium content of Bonanza shoot and root tissue as a function of EC and Ca/TC.

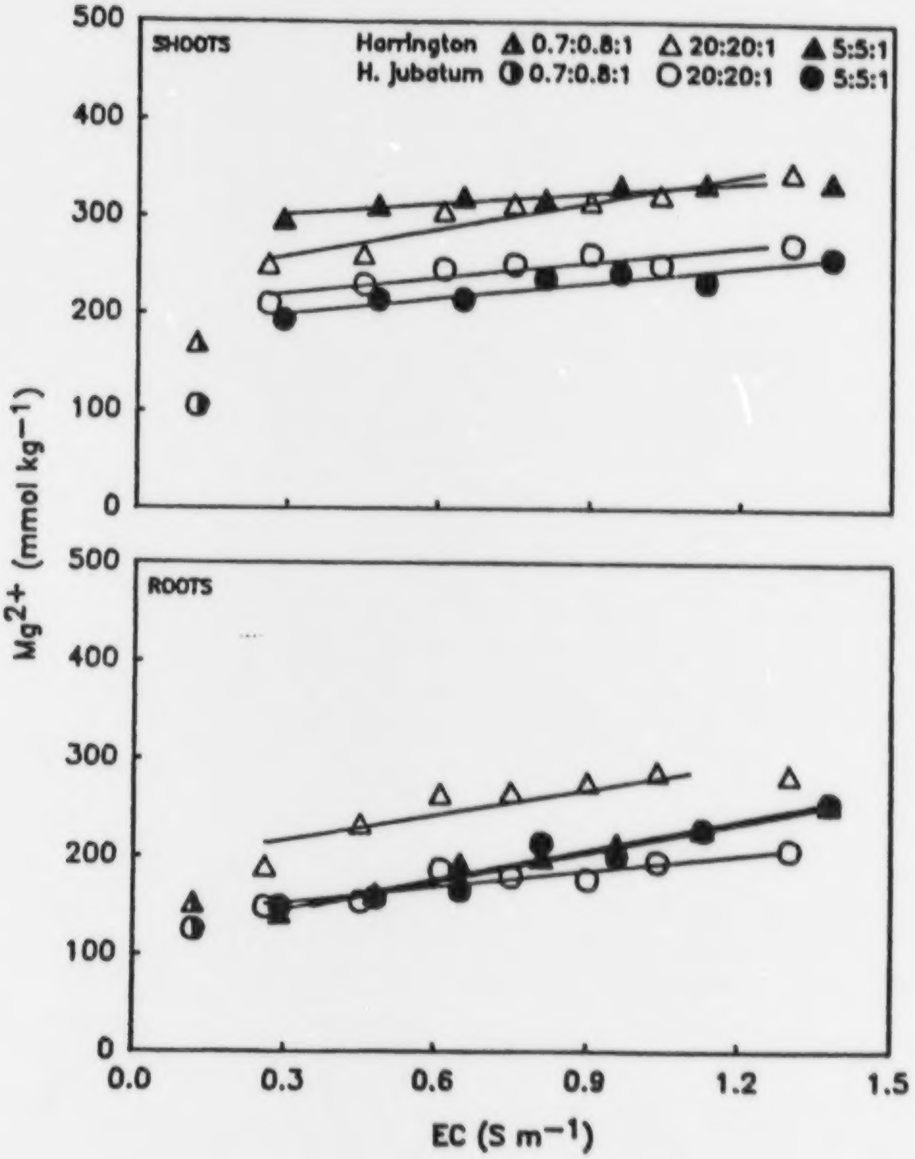

Fig. 3.10.1 Magnesium content of *H. jubatum* and Harrington shoot and root tissue as a function of EC and Ca/TC.

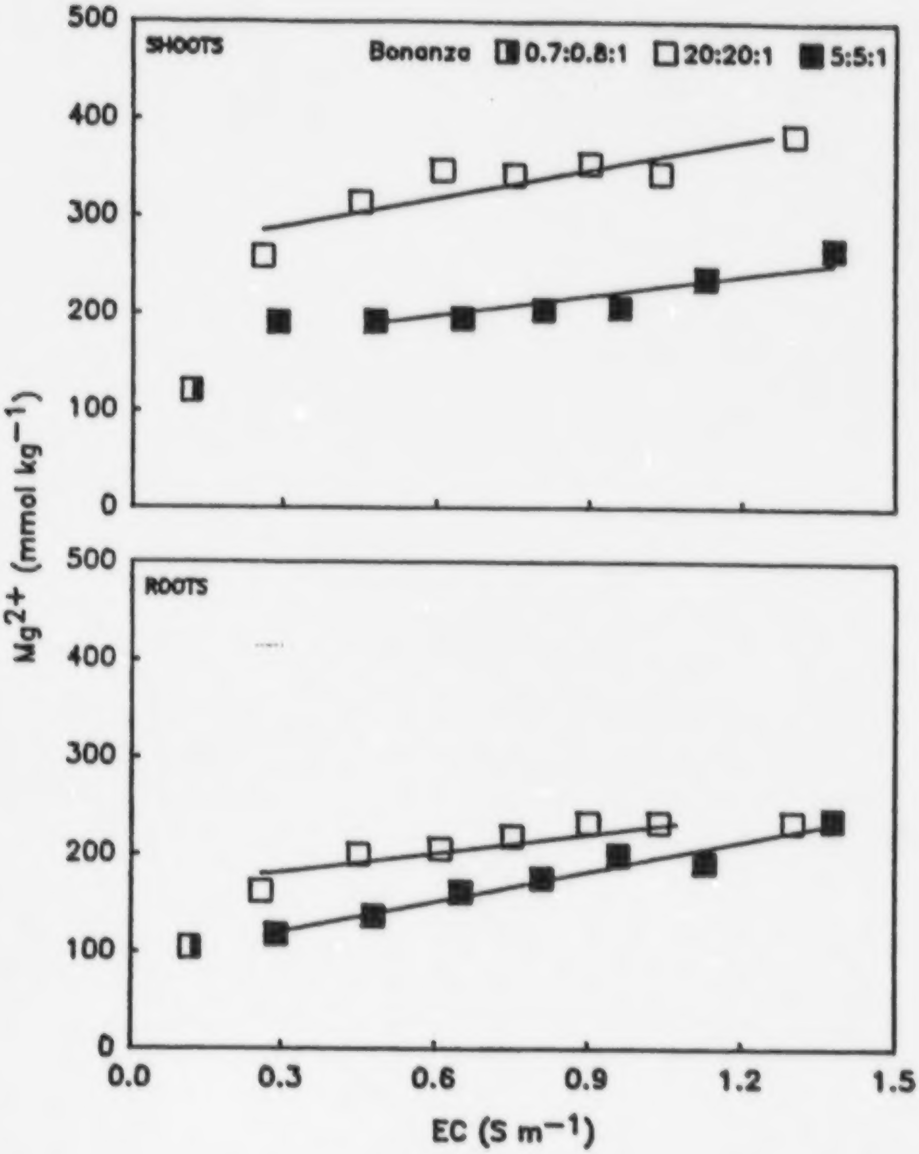

Fig. 3.10.2 Magnesium content of Bonanza shoot and root tissue as a function of EC and Ca/TC.

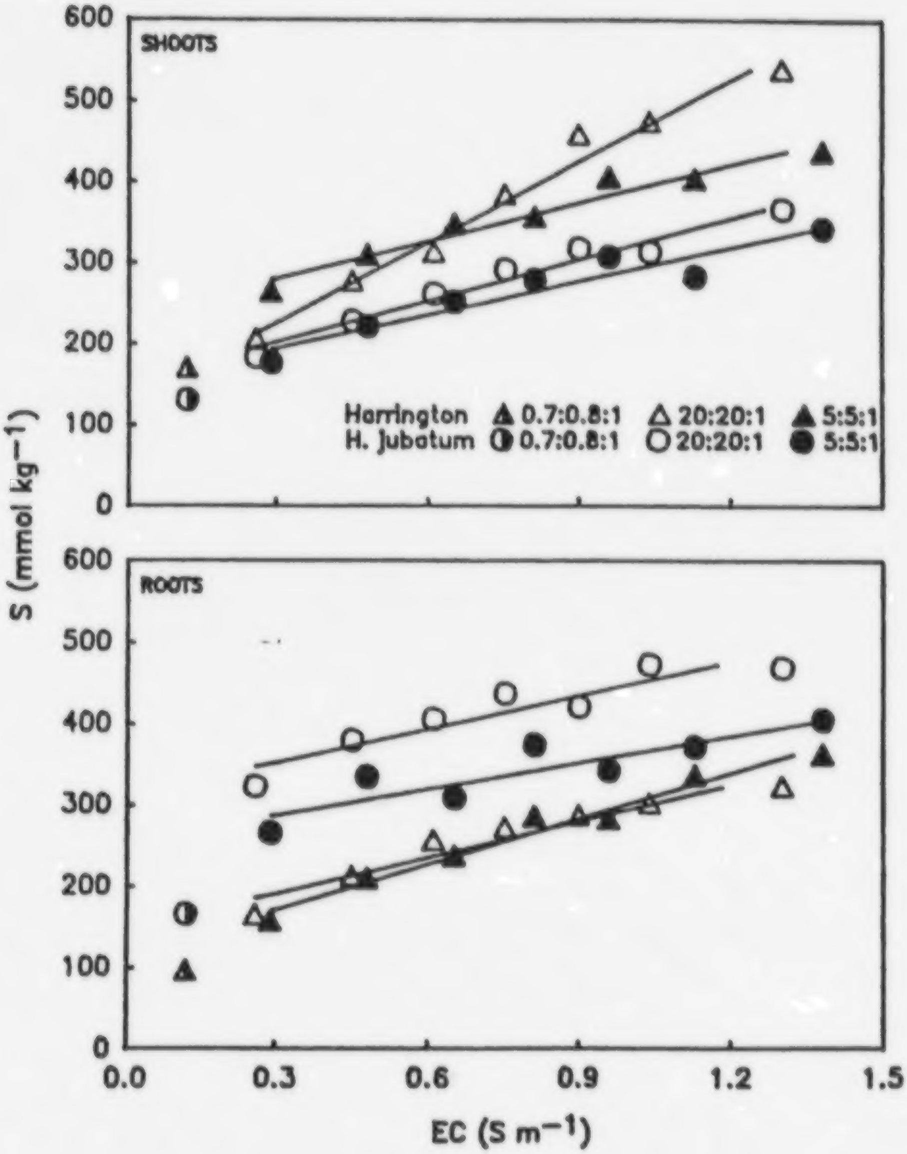

Fig. 3.11.1 Sulfate content of *H. jubatum* and Harrington shoot and root tissue as a function of EC and Ca/TC.

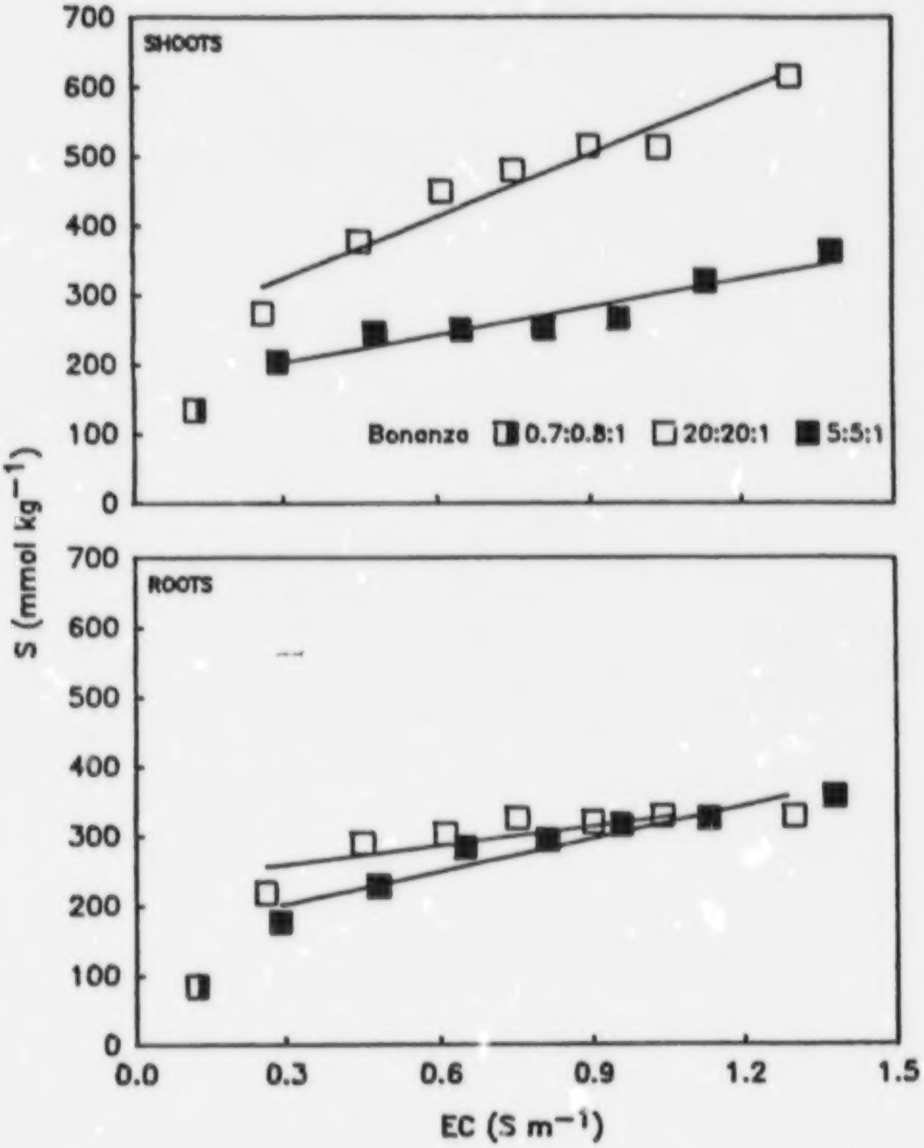

Fig. 3.11.2 Sulfate content of Bonanza shoot and root tissue as a function of EC and Ca/TC.

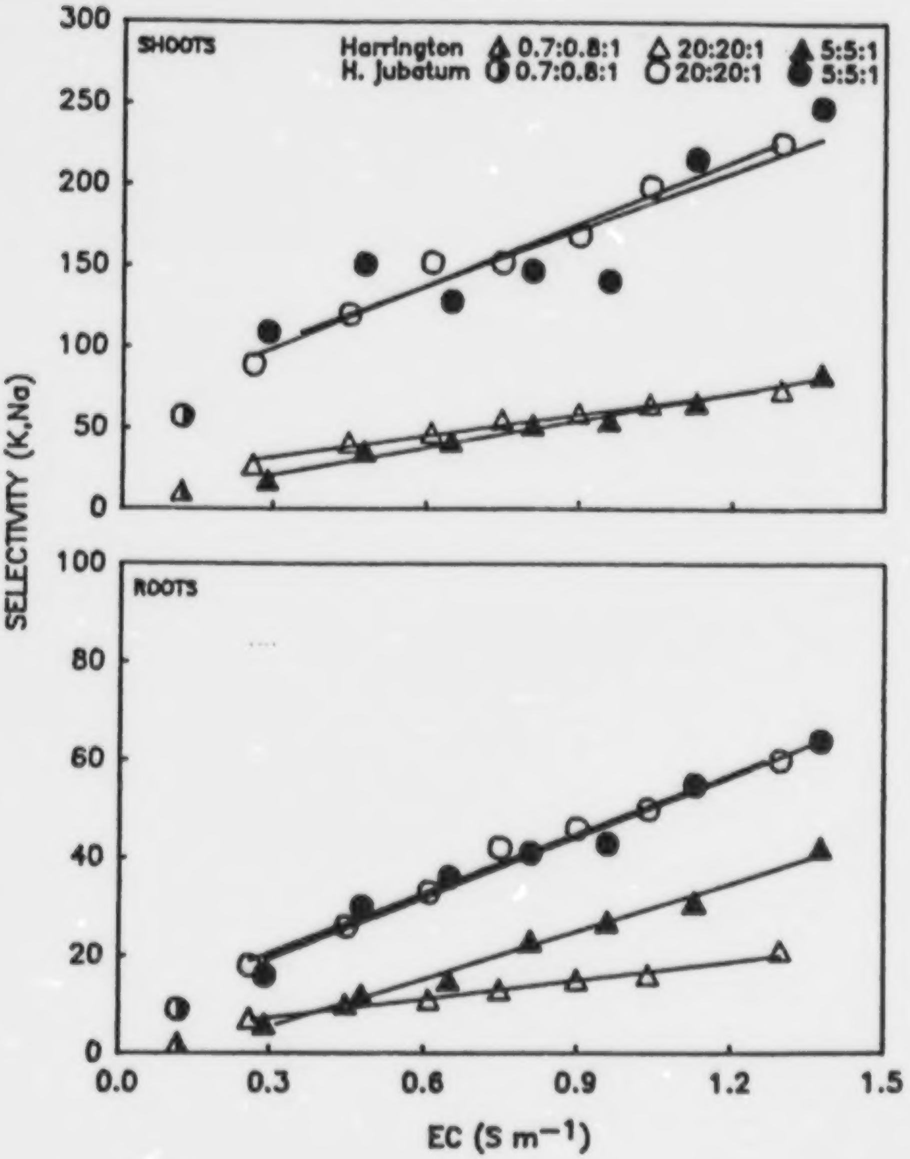

Fig. 3.12.1 Selectivity ($S_{K,Na}$) of *H. jubatum* and Harrington root and shoot tissue as influenced by increasing EC and the Ca/TC.

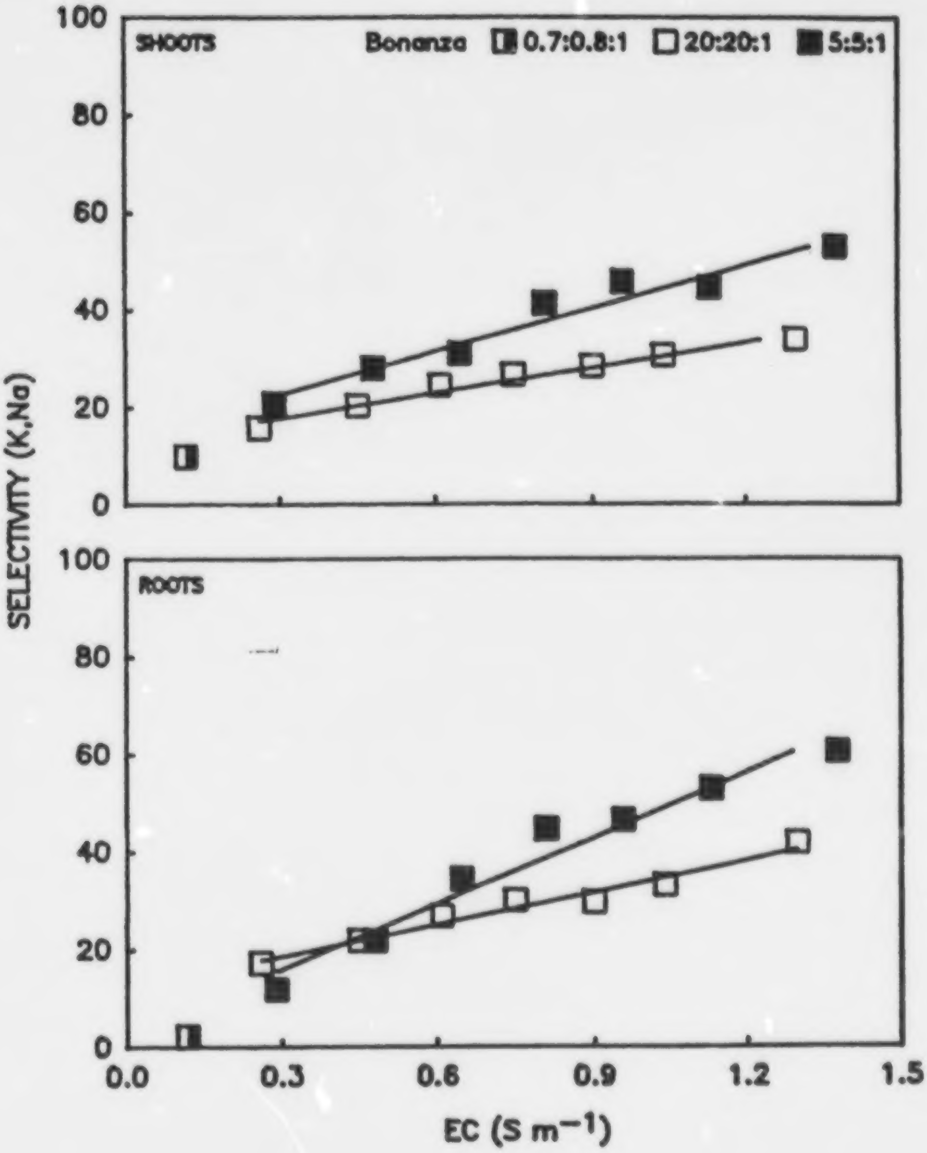

Fig. 3.12.2 Selectivity ($S_{K,Na}$) of Bonanza root and shoot tissue as influenced by increasing EC and the Ca/TC.

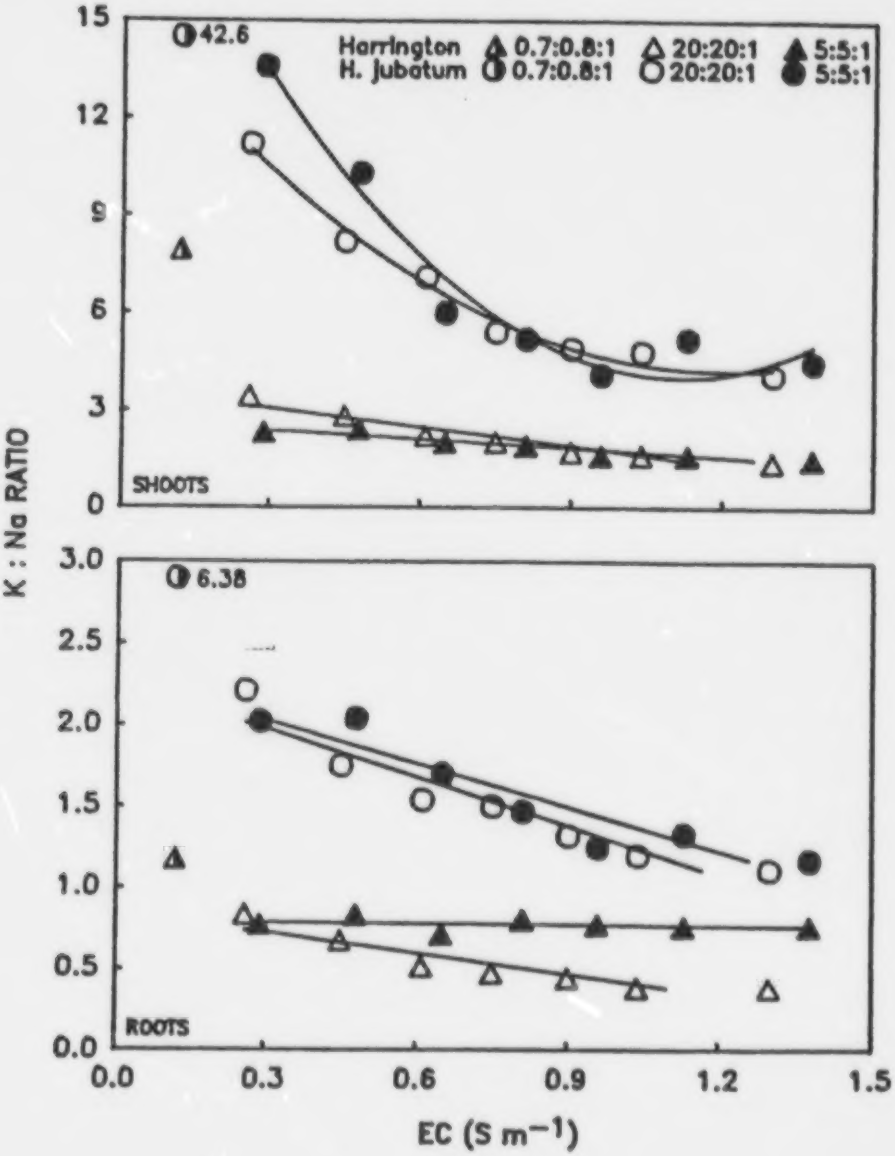

Fig. 3.13 Potassium:sodium ratio of *H. jubatum* and Harrington root and shoot tissue as influenced by increasing EC and the Ca/TC.

Harrington plants are presented in Fig. 3.13. *H. jubatum* maintains higher K:Na ratios than Harrington, that decay curvilinearly across the range of salt treatments in the wild species but not the cmmercial cultivar. An increase in the Ca/TC increases the K/Na in Harrington roots only; K/Na in *H. jubatum* was independent of Ca/TC.

3.4 Discussion

Salt-induced reductions in dry matter productivity, leaf area, and R/S of Harrington and Bonanza plants were more profoundly influenced by the Ca/TC of the growth medium than by solution EC (Fig. 3.4, 3.5, 3.6). Salinity stress caused a greater inhibition of root rather than shoot growth. Whereas shoot dry mass was raised to control levels for plants salinized at the higher Ca/TC, root dry mass fell short of control levels when the Ca/TC was increased from 0.02 to 0.07. Calcium/total cation ratios have been shown to significantly influence the dry matter production and salt tolerance of several agronomically important grass species. For example, *H. vulgare* cv. Galt required a Ca/TC of at least 0.15 for maximum yield (Carter et al., 1979). Shoot growth of *Zea mays* cv. DeKalb was substantially improved when Ca/Na ratios, added iso-osmotically, were raised from 0.03 to 0.18 (Maas and Grieve, 1987). Similar Ca/Na ratio effects on shoot growth were reported in salinity studies with several sorghum (Grieve and Maas, 1988) and rice genotypes (Grieve and Fujiyama, 1987). Our data show that these growth parameters for the wild barley species, *H. jubatum*, are not significantly influenced by salinity stress at a Ca/TC of

0.07 and only minimally affected at a ratio of 0.02 as compared to the commercial lines. This suggests the presence of a more efficient Ca uptake/utilization mechanism within wild species roots that affectively ameliorates salinity stress effects upon root growth inspite of unfavorable Ca:competitive cation ratios.

Genotypic differences in the R/S exist between the barley cultivars and the wild species. In control nutrient solutions at a Ca/TC of 0.32 the R/S for *H. jubatum* plants was 33% greater than the commercial lines (Fig. 3.6). Generally speaking, high R/S ratios are characteristic of plants having low per weight root absorption capacity, and low relative growth rates (Chapin, 1980). Salt treatments decreased the R/S in Harrington and Bonanza plants primarily through reductions in root dry mass; these cultivars displayed a strong dependence of the R/S on the Ca/TC of the solution. Values for R/S ratios of foxtail barley plants, across the range of salt treatments, were larger in magnitude (or as % control) and less responsive to Ca/TC as compared to Harrington and Bonanza. Our data point to a strong inverse correlation between R/S and the Na content of salt stressed root tissue. Similar relations between R/S and monovalent cation absorption have been reported for the marsh halophyte, *Spergularia marina* (Cheeseman and Wickens, 1986) and for *H. vulgare* cv. Villa (Jeschke, 1982). In the commercial lines a decrease in R/S is accompanied by a sharp increase in root Na concentrations; this relation being modulated by the Ca/TC of the stress medium. *H. jubatum* plants on the other hand, maintain high R/S and low levels of Na in root tissue. The R/S ratio might serve as one selection parameter useful in the determination of the relative salt tolerance of barley species.

Ion uptake and allocation between roots and shoots was strongly influenced by the Ca/TC in the commercial lines with all three genotypes showing the same pattern of translocation for Ca in the plant (Fig. 3.9.1, 3.9.2). In a more Ca rich medium Ca ions were preferentially transported to and stored in shoots rather than roots. However, root Ca levels for *H. jubatum* were 2-fold greater than Ca in Harrington and Bonanza roots.

The relationship between ion uptake/compartmentation in plant tissue and the Ca level of the medium is complicated by the use of a complex salt stress consisting of both monovalent (Na) and divalent (Mg) cations. The interaction between these cations and preferred ligands such as would be located on root cell walls or membranes would be governed by electrostatic forces. Sodium ions primarily screen charges whereas Mg ions bind to and screen charges at negatively charged membrane surfaces (McLaughlin et al., 1971). Thus Na and Mg ions would compete differently for Ca specific ligands.

Higher levels of Ca in the salt solutions, expressed as Ca/TC, increased $S_{K,Na}$ in root tissues of Harrington and Bonanza, and Bonanza shoot tissue. Similar Ca/TC-dependent increases in $S_{K,Na}$ and K/Na ratio have been reported for *H. vulgare* cv. Galt (Carter et al., 1979) and sorghum (Grieve and Maas, 1988). It is noteworthy that root ion selectivity for Harrington and Bonanza increase without an accompanying real increase in root Ca levels. This suggests that Ca ions may serve a protective role in the root apoplastic space or at the outside face of the root cell plasma membrane. At the membrane surface Ca ions might protect important, salt sensitive components of membrane-bound ion tranport systems, that may be mediated at the level of the root cell plasma membrane. The inherently greater levels of Ca in *H. jubatum* roots may in part be responsible for the consistently greater $S_{K,Na}$ values for the wild species over the commercial lines with plants salinized at Ca/TC of 0.02. Roots from Bonanza plants salt stressed at Ca/TC of 0.07 had $S_{K,Na}$ equal to the wild species across the range of salt treatments (Fig. 3.12.1, 3.12.2). These observations again suggest a Ca-mediated role for membrane permeability/integrity. The superior $S_{K,Na}$ of wild species roots over the commercial cultivars at low Ca/TC salt stress indicate the existence of functional and/or structural differences of root membrane ion transport properties of *H. jubatum* plants.

The greater ability of foxtail barley plants to exclude Na from shoots (Fig. 3.7.1) and the curvilinear response of K/Na with increasing salinity in shoot tissue as compared with Harrington (Fig. 3.13) suggest that differences in Na ion loading into xylem in the root, or phloem retranslocation of Na from shoot to root may exist between these species. The reabsorption of ions from xylem vessels, a salt exclusion mechanism present in many legume species, does not appear to be present in barley species (Pitman, 1984). In *H. distichon* roots a significant rate governing anion dependence has been demonstrated for Na transport and Na loading into root xylem vessels, with rate decreasing in the order $NO_3^- > Cl^- >> SO_4^{2-}$ (Jeschke, 1980). The higher levels of S (SO_4^{2-}) in Harrrington shoot tissue suggest that the ion transport system active in xylem loading in root tissue may be less discriminating with respect to anion requirement than the system present in *H. jubatum*, thus facilitating higher Na levels in xylem and resulting in greater Na accumulations in shoot tissue. Phloem transport of Na from shoot to root has been demonstrated in *Lupinus albus* subjected to mild salinity stress and it is suggested that this may have some importance in reducing the Na load in leaf tissue (Jeschke et al. 1987). Basipetal phloem transport of Na ions may be a mechanism of significance in salinity tolerance (Jeschke, 1984).

Sodium levels in root tissue of the two commercial lines are governed by the Ca/TC of the solution and are accumulated to higher levels than in *H. jubatum* roots (Fig. 3.7.1, 3.7.2). On the other hand, *H. jubatum* accumulates more K in root tissue than Harrington; in Harrington roots K--accumulation is sensitive to Ca/TC (Fig. 3.8.1). These data strongly suggest structural and/or functional differences in the root cell plasma membrane between commercial and wild species that influence Na and K entry and retention in the cytoplasm. The structural basis for the greater K and the lower Na permeability of *H. jubatum* versus Harrington roots might be attributed to 1) the membrane potential-dependent influx of K through a specific ion channel with an affinity for K>>Na, 2) the proton-dependent extrusion of Na from the cytoplasm to the cell exterior via a Na^+-H^+ antiporter (Jeschke, 1984).

H. jubatum plants maintained the lowest Mg levels in roots and shoots under low Ca/TC salinity conditions (Fig. 3.10.1, 3.10.2). The Mg levels in Bonanza shoots, and Bonanza and Harrington roots showed a downward displacement at the higher Ca/TC stress regime that suggests competition between Mg and Ca for uptake and translocation in the commercial lines. Previous reports have shown that Mg/Ca ratios greater than 2.5 in salinized nutrient solutions adversely affect yield of *H. vulgare* cv. Galt (Carter et al., 1979).

Ion accumulation and compartmentation in root and shoot tissues may be an energy efficient and viable alternative to synthesis of organic solutes for osmotic adjustment in root and shoot tissues of plants subjected to salinity stress (Yeo, 1983). However the use of inorganic ions for osmotic adjustment in the plant can only be feasible if 1) favorable K/Na ratios are maintained, 2) monovalent ion accumulation does not interfere with normal Ca nutrition, 3) there is a proper compartmentation of cations and anions accumulated. *H. jubatum* plant growth as opposed to Harrington and Bonanza is relatively uneffected by the salt stress treatments imposed in our experiments. The greater salt tolerance of foxtail barley as opposed to the commercial lines might be attributed to 1) a more efficient use of Ca under salinity stress, 2) the maintainence of favorable K/Na ratios, and 3) a more efficient use of anions i.e. SO_4^{2-}, for osmotic adjustment in root tissues. Taken as a whole, the ion analysis data suggest that the differences in salt tolerance between *H. jubatum* and the commercial lines, may well be attributed to differences in ion transport properties at three key points: 1) K-Na discrimination at the root plasma membrane, 2) cation-anion loading of root xylem vessels, and 3) phloem-mediate basipetal transport of Na from shoot to root. Certainly these three areas require further investigation so as to assess their importance in the overall salinity tolerance of barley species.

3.5 Summary

The relative salt tolerance of commercial and wild barley species was evaluated as a function of increasing salt concentration (Na_2SO_4, $MgSO_4$, and $CaCl_2$), and the calcium/total cation ratio (Ca/TC) in solution culture experiments. The wild barley species, *H. jubatum*, was superior in salt tolerance to *H, vulgare* cv. Harrington, and cv. Bonanza as measured by growth parameters. Salinity treatments imposed at a Ca/TC of 0.02 severely affected root growth and reduced the root:shoot (R/S) in both commercial lines, whereas *H. jubatum* was only minimally affected. These same growth parameters in Harrington and Bonanza improved substantially when the Ca/TC was raised from 0.02 to 0.07, while this shift in Ca/TC resulted in *H. jubatum* plants that were essentially equivalent to controls in growth.

An examination of the ion relations of these three species revealed that *H. jubatum* plants maintained higher levels of Ca, had more favorable K/Na ratios, and maintained higher R/S levels of SO_4^{2-}, than the commercial lines. These differences in ion translocation between *H. jubatum* and the commercial cultivars suggest that the wild species maintains superior membrane permeability properties even under conditions where plants are salinized at low Ca/TC. The superior ion selectivity and ion exclusion properties of *H. jubatum* plants may be linked to transport properties associated with: the root plasma membrane, root xylem loading of ions, and possibly, phloem retranslocation of ions from shoots to roots.

Our study suggests that *H. jubatum* may be a useful source of salt tolerant germplasm with potential for integration into *H. vulgare* through a breeding or molecular genetic transfer program.

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4.0 SALINITY EFFECTS ON CELL PHYSIOLOGY AND MEMBRANES

4.1 Introduction

The cell plasma membrane and the various subcellular organelle-associated membranes function as semipermeable barriers to ion and solute movement. Membranes regulate the uptake, translocation, and compartmentation of ions in plant tissues via a series of membrane-bound transport proteins that function as ion channels. Transport is driven at the expense of the electrochemical H^+-gradient that is generated by H^+-pumping ATPases located on the plasma membrane and other subcellular membranes (endoplasmic reticulum, Golgi, tonoplast) of the plant cell (Sze, 1985). The transport of essential ions like K^+ from the cell exterior into the cytoplasm is driven by the membrane potential (inside negative) generated by active H^+ extrusion at the plasma membrane (Serrano, 1989). The long distance transport of ions and solutes in tissues via the xylem and phloem, require H^+-pump dependent active transport processes for xylem and phloem loading (Serrano, 1989).

Jeschke has proposed that ion selectivity at the root level and for the plant as a whole is determined by the ion selectivity of three membranes: the plasma membrane of root cortical cells and root xylem parenchyma, and the tonoplast membrane of root and shoot cells (Jeschke, 1984). The plasma membrane of root cortical cells serves as the primary barrier to cytoplasmic ion accumulation in roots subjected to a saline rhizosphere. Transient fluxes of divalent and monovalent cations influence membrane electrostatic properties (Caldwell et al., 1983, McLaughlin et al., 1971) and membrane viscosity (Caldwell et al., 1983, Suhayda and Haug, 1986). Prolonged exposure to salinity stress has been shown to induce permanent changes in the electrostatic properties of the tomato root plasma membrane (Suhayda et al. 1990).

In light of the data presented in Chapter 3.0 on whole plant growth and ion accumulation, the objective of the present study was to examine the effects of a steady state salinity stress ($MgSO_4$, Na_2SO_4, $CaCl_2$) on the H^+ transport properties and the surface electrostatic properties of *H. vulgare* cv. Harrington root cell plasma membrane. Our longer term goals are 1) to characterize what differences, if any, exist between the ion transport properties of root plasma membrane preparations isolated from salinized commercial and wild barley species, and 2) to correlate membrane transport properties with the ion uptake and distribution patterns observed in salt stressed commercial and wild barley species.

4.2 Methods

H. vulgare cv. Harrington plants were grown and salinized under the conditions described in section 3.2. The two salt treatments used were Mg:Na:Ca=20:20:1 (Ca/TC = 0.02, EC = 0.9 S/m) and Mg:Na:Ca=5:5:1 (Ca/TC = 0.07, EC = 0.96 S/m); see treatment 6, Table 3.1.

4.2.1 Plasma membrane isolation.

The plasma membrane-enriched vesicle fractions that were used for ion transport measurements were isolated by the method of Giannini et al. (1987). All solutions were kept at ice water temperature, and all procedures were performed on ice. Excised barley roots were briefly rinsed with deionized water, blotted to remove excess water, cut into small segments, and immediately placed into homogenizing buffer. This buffer consisted of 70 mM Tris-HCl (pH 8.0) with 250 mM sucrose and contained the following: 10% (v/v) glycerol, 3 mM EDTA, 2 mM PMSF, 4 mM DTE, 15 mM beta-mercaptoethanol, 2 mM Na_2ATP, and 250 mM KI. Prior to hand homogenization by mortar and pestle the root tissue was vacuum infiltrated for 5 min with homogenizing buffer. The homogenate was filtered through 4 layers of cheesecloth and then centrifuged for 15 min at 13,000g using a Sorvall SS-34 rotor. The resulting supernatant was centrifuged at 85,000g using a Beckman SW 28 rotor for 30 min. The supernatant was discarded and the microsomal pellet was carefully resuspended by gentle homogenization with a ground glass homogenizer in a buffer consisting of 2 mM BTP-Mes (pH 7.0) with 250 mM sucrose and containing the following: 10% (v/v) glycerol, 1 mM DTE, 1 mM PMSF. For routine preparation of plasma membrane-enriched fractions, microsomes were layered onto a discontinuous sucrose gradient (34%/40%) and centrifuged in a Beckman SW 28 rotor at 85,000g for 2h. The plasma membrane fraction was recovered from the 34%/40% sucrose interface and the samples were either used immediately or frozen in liquid N_2 and stored at -80°C.

4.2.2 Measurement of H^+-transport and ATPase activity.

Proton transport activity was determined by measuring H^+ accumulation within the plasma membrane vesicles upon MgATP addition. This process was monitored by the rate of quench of the pH-sensitive fluorescent probe, quinacrine. Fluorescence measurements were recorded with a SPEX Model 112A spectrofluorimeter at 28°C. Excitation and emission wavelengths for quinacrine were 430 and 500 nm, respectively. Excitation and emission slits were 1.25 mm. The vesicles were assayed by placing 50 ug membrane protein into a buffered solution consisting of 25 mM BTP-Mes (pH 7.0) with 250 mM sorbitol and containing the following: 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), and 200 mM monovalent ions as indicated in results. For all assays, quinacrine was present at a final concentration of 2.5 uM, and the steady state quench (%Q/mg protein) determined after the addition of 5 uM gramicidin (Briskin et al., 1985).

ATPase assays were performed in 25 mM BTP-Mes (pH 6.5) buffer containing 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), and 5 uM gramicidin. Vanadate-sensitive, plasma membrane-bound ATPase activity was determined by the difference in activity between assays supplemented with 50 mM KCl or 50 mM KCl plus 100 uM sodium orthovanadate (Poole et al., 1984). Tonoplast activity was determined from the difference in activity measured in assays containing 50 mM KCl and those with 50 mM KNO_3 (Poole et al., 1984). Mitochondrial membrane fractions were localized using the cytochrome C oxidase assay.

The colorimetric assay of Peterson (1977) was used to determine membrane protein concentration using BSA as the protein standard. Inorganic phosphate was measured by the colorimetric method of Peterson (1978).

4.2.3 Measurement of membrane surface potential.

Plasma membrane vesicles were titrated with the anionic, fluorescent probe ANS (8-anilino-1-napthalene sulfonic acid) obtained from Kodak. Surface potentials were estimated by the method of Gibrat et al. (1983). Excitation and emission wavelengths for the dye were 360 and 480 nm, respectively, and cutoff filters were used to minimize scattered light. Titrations with ANS were made in a buffered system (25 mM BTP-Mes, pH 7.0, 0.25 M sorbitol) by successive additions of dye to the vesicle suspension (75 ug protein/3 ml). Following a 1 min dye equilibration period, data were collected by signal averaging for 2 min.

4.3 Results

4.3.1 Isolation of plasma membrane-enriched vesicles.

Microsomal membrane fractions obtained by differential centrifugation from control barley roots were separated on sucrose density gradients and the data is presented in Table 4.1. This isolation procedure yields inside-out, H^+-transport competent plasma membrane vesicles (Fig. 4.1). Following sucrose density gradient centrifugation of the crude microsome fraction (85,000g pellet), approximately 20% of the vanadate-sensitive, KCl-stimulated, MgATPase activity was recovered from the 34%/40% sucrose interface. This band contains the plasma membrane enriched fraction. There was no NO_3^--sensitive activity (tonoplast membrane marker), and little cytochrome C oxidase activity (mitochondrial membrane marker) associated with the plasma membrane fraction.

Table 4.1 *Distribution of marker enzymes in membrane fractions isolated from Harrington root tissue*

Fraction x g	Protein mg	% total	Vanadate-sensitive, K-MgATPase umol P_i/hr	% total
13,000 pellet	27.9	22.0	247.8	10.1
13,000 supernatant	98.7	78.0	2,203.0	89.9
85,000 pellet	14.3	11.3	446.2	18.2
85,000 supernatant	86.6	68.4	1,482.6	60.5
Sucrose gradient:				
Overlay	3.8	3.0	121.0	4.9
34% sucrose	2.6	2.1	91.5	3.7
34%/40% interface	2.4	1.9	91.6	3.7
40% sucrose	3.4	2.7	38.9	1.6
Pellet	3.3	2.6	44.1	1.8

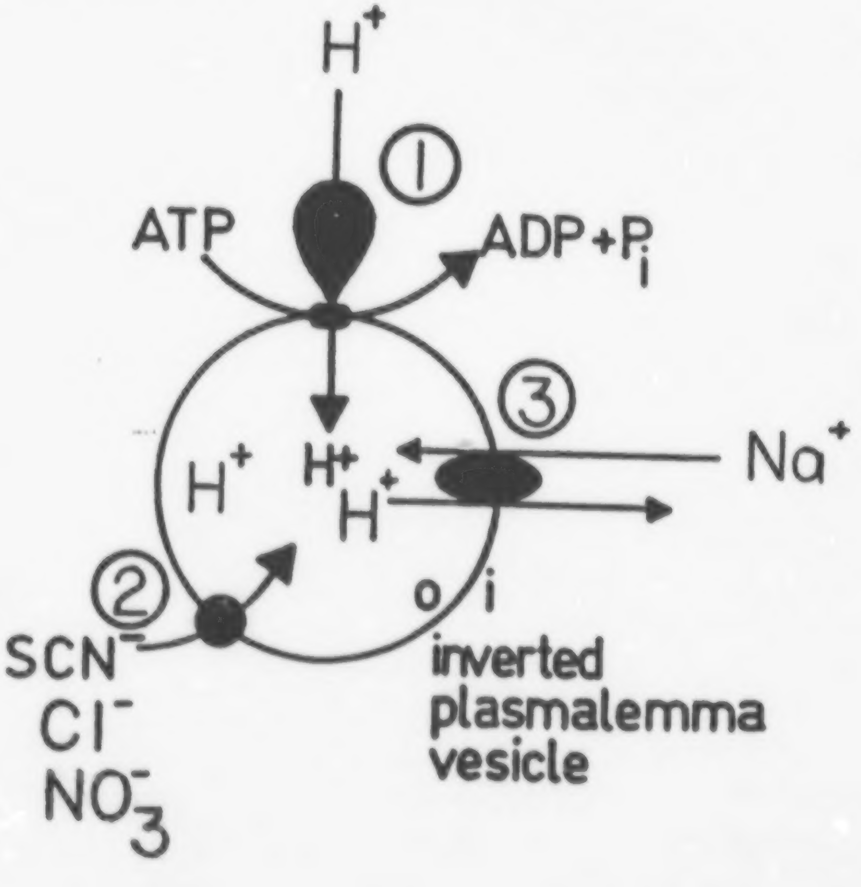
Fraction x g	Cytochrome C Oxidase umol cyt C/min	% total	NO_3^--sensitive, K-MgATPase umol P_i/hr	% total
13,000 pellet	2.57	46.4	75.9	22.1
13,000 supernatant	2.96	53.5	268.5	78.0
85,000 pellet	1.85	33.5	57.2	17.0
85,000 supernatant	0.62	11.1	0	0
Sucrose gradient:				
Overlay	0.07	1.2	33.1	9.6
34% sucrose	0.15	2.6	0	0
34%/40% interface	0.24	4.2	0	0
40% sucrose	0.23	4.2	2.2	0.6
Pellet	0.79	14.3	4.2	1.2

Fig. 4.1 Inverted plasma membrane vesicle system used of H^+-transport measurements.

(1) plasma membrane-bound, H^+-pumping ATPase

(2) anion channel

(3) Na^+/H^+ antiporter


H^+
①
ATP
ADP+P_i
③
Na^+
H^+
H^+
H^+
②
SCN^-
Cl^-
NO_3^-
o
i
inverted plasmalemma vesicle

These results along with a previous report on barley plasma membrane (DuPont et al., 1988) indicate that sucrose gradients can be used to provide H^+-transport competent plasma membrane vesicles.

4.3.2 H^+-transport properties of plasma membrane fractions from salt stressed barley roots.

Membrane vesicles were isolated from roots stressed at 1) a Ca/TC ratio (0.07) where the solution electrolyte conductivity was 0.96 S/m, and the ratio of salts used in the applied stress was Mg:Na:Ca=5:5:1, and 2) a Ca/TC ratio (0.02) where the solution EC was 0.90 S/m, and the ratio of salts used in the applied stress was Mg:Na:Ca=20:20:1. Differences in the MgATP-dependent rate of H^+-gradient formation and the magnitudes of the H^+-gradients formed were apparent between the plasma membrane vesicles isolated from roots salinized with these two salt treatments (Fig. 4.2). Membranes isolated from roots stressed at the higher Ca/TC had greater KCl and NaCl-dependent rates of H^+-transport than vesicles isolated from roots salinized at the lower Ca/TC ratio. In both types of plasma membrane preparations however, the KCl-stimulated H^+-transport was greater both in rate and magnitude (measured 5 min after initiation of reaction with MgATP) than that stimulated by NaCl. A comparison of the steady state quench values determined in the presence of KCl, NaCl, or Cl^- (Table 4.2), indicates that the presence of Na^+ ions in the assay medium reduces the buildup of H^+ within the plasma membrane vesicles. In all cases the addition of 5 uM gramicidin, a H^+ ionophore, released the H^+-gradient from within the vesicles. This demonstrates that the assay measures a

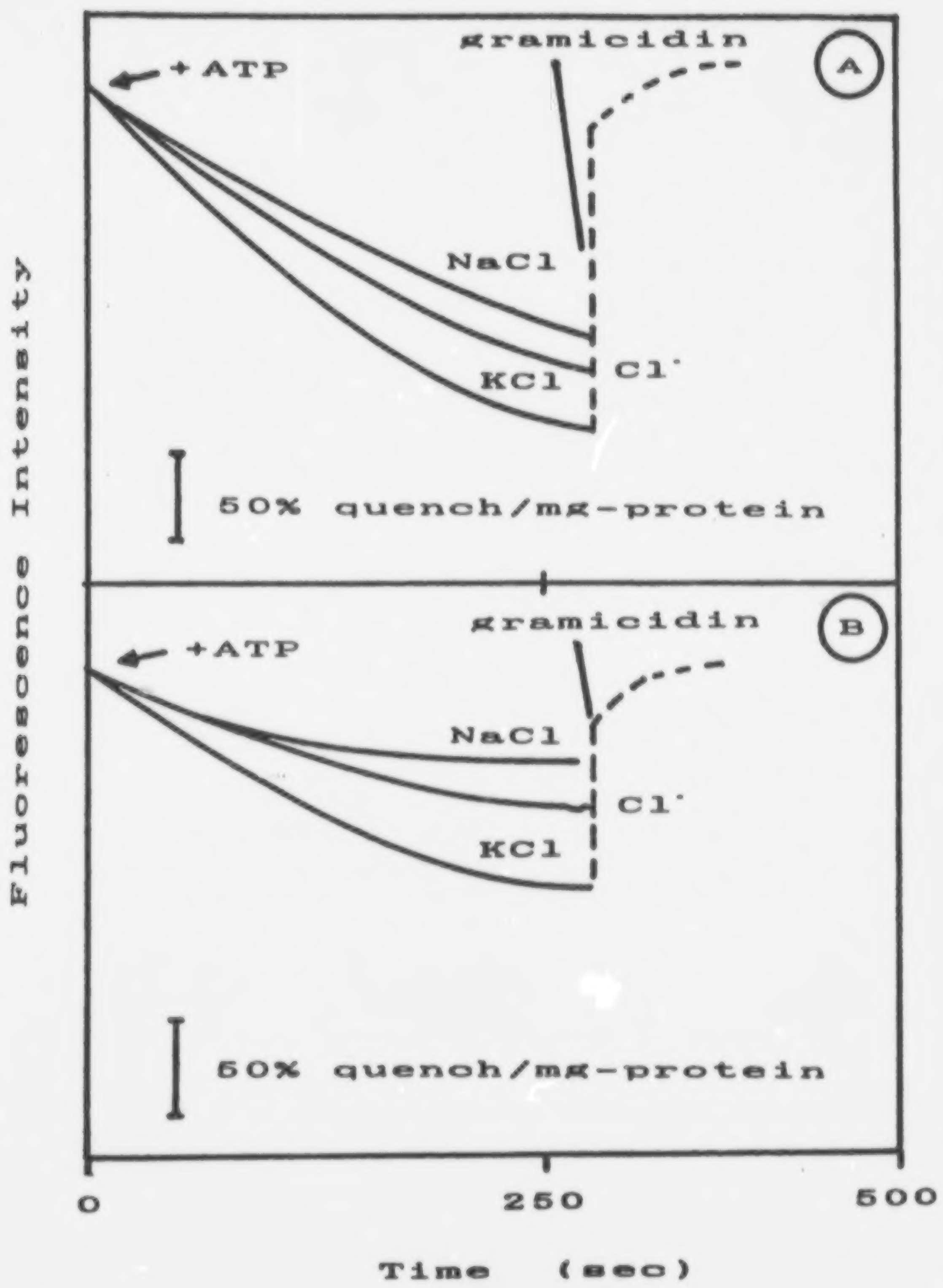


Fig. 4.2 Quinacrine quench assay used to measure H^+-transport into Harrington plasma membrane vesicles. (A) activity of vesicles isolated from roots stressed at Ca/TC=0.07, Mg:Na:Ca=5:5:1. (B) activity of vesicles isolated from roots stressed at Ca/TC=0.02, Mg:Na:Ca=20:20:1. Ions were added at a final concentration of 200 mM; ATP and

Table 4.2 *Proton transport activity in plasma membrane vesicles isolated from salt stressed Harrington root tissue.*

	Mg:Na:Ca=5:5:1	Mg:Na:Ca=20:20:1
Assay mixture	% Quench/mg-protein	% Quench/mg-protein
MgATP + 200 mM Cl^-	187	89
MgATP + 200 mM KCl	244	129
MgATP + 200 mM NaCl	158	50
MgATP + 200 mM KNO_3	275	166
MgATP + 200 mM KCl 100 uM vanadate	170	87

Table 4.3 *Apparent and intrinsic dissociation constants for ANS binding and estimated surface potentials for plasma membrane vesicles isolated from control and salt stressed Harrington root tissue.*

	Apparent Dissociation Constant K_d (uM)			Surface Potential Ψ_o (mV)		
KCl (M)	Con	Ca/TC=.02	Ca/TC=.07	Con	Ca/TC=.02	Ca/TC=.07
0	85	82	79	-14.0	-14.3	-15.3
0.15	79	73	72	-12.0	-11.4	-13.2
1.5	58	55	52	-4.2	-4.0	-4.6
K_i =	49	47	43			

Note: Where Ca/TC=0.02, the ratio of salts in the applied stress was Mg:Na:Ca=20:20:1
Where Ca/TC=0.07, the ratio of salts in the applied stress was Mg:Na:Ca=5:5:1

true H^+-gradient rather than nonspecific dye-vesicle interactions (Briskin et al., 1985).

Proton transport was stimulated by anions with NO_3^- enhancing H^+ accumulation more than Cl^-: compare KCl, KNO_3, and Cl^- stimulation in Table 4.2. H^+-transport was inhibited by vanadate, an inhibitor of plasma membrane ATPase, but not NO_3^-, a tonoplast ATPase inhibitor (Table 4.2). The inhibitor data also indicate that the vesicle fraction used in these experiments is primarily of plasma membrane origin.

4.3.3 Membrane surface potential determination.

ANS has been used to effectively estimate the microscopic surface potential (Ψ_0) of plant and mammalian membranes (Chiu et al., 1980, Gibrat et al., 1983, Gibrat et al., 1985). Dye binding to the membrane results in the enhancement of ANS fluorescence and the rapid partitioning ($t_{1/2}$ about 8 sec) of the dye between the outer and inner membrane surface (Chiu et al., 1980).

Titration of control and salt-stressed plasma membrane vesicles in the presence of 0, 0.15, and 1.5 M KCl resulted in fluorescence curves displaying saturation kinetics from which the dissociation constants (K_d) were determined (Table 4.3). Determination of K_d under two salt conditions allowed for the calculation of an intrinsic binding constant (K_i) using the equation (Gibrat et al., 1983):

$$K_i = \frac{(K_d)_1 \sqrt{r[(K_d)_2/(K_d)_1]} - (K_d)_2}{\sqrt{r[(K_d)_2/(K_d)_1]} - 1}$$

where $r = E_1 \cdot [KCl]_1 / E_2 \cdot [KCl]_2$ and E_1 and E_2 are the dielectric constants of the salt solutions. In this case K_i represents the dye binding constant under conditions where Ψ_0 is zero. Determination of K_d and K_i allows for the calculation of Ψ_0 from (Gibrat et al., 1983):

$$\Psi_0 = (kT/e) \ln K_i/K_d$$

where k is the Boltzmann constant, and e is the electronic charge. As shown in Table 4.3, no significant difference was found in surface potential values between control and salt stressed plasma membrane vesicles. Individual values for control membranes and membranes isolated from roots salt stressed at Ca/TC = 0.02 (Mg:Na:Ca=20:20:1), and Ca/TC = 0.07 (Mg:Na:Ca=5:5:1) were -14.0 mV, -14.3 mV, and -15.3 mV, respectively.

4.4 Discussion

The root cell plasma membrane serves as an important control point for the exclusion or selective uptake of essential ions into the cell using the electrochemical gradient generated by the H^+-pumping ATPase. With this in mind we examined the effects of excess salts in the growth medium on the activity of the plasma membrane-bound, H^+-pumping ATPase from salt stressed Harrington barley root tissue. In our inverted plasma membrane vesicle system, the addition of MgATP to the vesicle suspension initiates the transport of protons by the ATPase (#1, Fig. 4.1) from the medium into the vesicle lumen. This generates a pH (inside acid), and membrane potential (inside positive) gradient between the vesicle interior and the outside medium. Anions such as NO_3^- and Cl^- are permeable to the membrane (perhaps through an anion channel, #2, Fig. 4.1) and accumulate within the vesicle in response to the buildup of positive charge. The collapse of the membrane potential, resulting from the influx of anions, stimulates H^+-transport activity.

Our results indicate that plasma membrane vesicles isolated from roots salt stressed at the lower Ca/TC ratio (0.02) had less salt stimulated H^+-transport activity than vesicles isolated from roots stressed at the higher Ca/TC ratio (0.07) (Fig. 4.2, Table 4.2). This decreased activity was independent of the type of salt or ion added (KCl, NaCl, KNO_3, or Cl^-). Two explanations for this result could be 1) that there are less ATPase molecules (see #1, Fig. 4.1) in plasma membrane vesicles isolated from roots stressed at Ca/TC = 0.02, compared with those stressed at Ca/TC = 0.07, or 2) that there may be differences in the membrane lipid composition of vesicles; these compositional differences influencing membrane viscosity and thereby altering H^+-pump activity.

A second finding of interest is the NaCl stimulation of H^+-transport activity in the vesicles as compared to KCl and Cl^- (Fig. 4.2, Table 4.2). The data show that H^+-pump activity in the prsence of 200 mM NaCl is not only less than that observed with 200 mM KCl but actually less than that found with 200 mM Cl^- alone. In terms of the model presented in Fig. 4.1, this result suggests that there is active Na^+/H^+ exchange occurring via an antiporter (#3, Fig. 4.1) at the expense of the H^+-gradient generated by the ATPase. The activities of plasma membrane vesicles expressed as the ratio of $NaCl/Cl^-$ stimulation of H^+-pumping had values of 56% and 84% for membranes isolated from roots salinized at Ca/TC = 0.02 and 0.04, respectively. This suggests that membranes salt stressed at the lower Ca ratio (Mg:Na:Ca=20:20:1) have more Na^+/H^+ exchange activity and thus perhaps more antiporter molecules on the plasma membrane than membranes salinized at the higher Ca ratio (Mg:Na:Ca=5:5:1).

Membrane surface potential measurements indicate that under the salinity stress conditions imposed in our experiments there was no significant difference in this membrane property between control and salt stressed plasma membrane vesicles (Table 4.3). It is interesting to note that barley, a more salt tolerant plant had a ψ_0 value for control Harrington plasma membrane of -14.0 mV whereas the reported values measured for corn and tomato root plasma membranes were -20.0 mV (Gibrat et al., 1985) and -26.0 mV (Suhayda et al., 1990), respectively. For tomato a decrease in the surface potential from -26.0 mV to -13.7 mV was found in root plasma membrane fractions isolated from control and salt stressed roots respectively (Suhayda et al., 1990). Apparently salinized barley root plasma membranes do not respond in a similar fashion.

Taken together these data indicate that salinity may have wide ranging effects on the structural and functional properties of the barley root plasma membrane. Our study indicates salinity stress at a low Ca/TC induces differences in H^+-transport activity and Na^+/H^+ activity in Harrington root plasma membranes. Additional work is required to establish a structural basis for these observed functional changes. In this respect, we have undertaken a new study to examine differences in the protein compostion of control and salinized Harrington root tissue in an effort to quantitate possible differences in plasma membrane associated ATPase and Na^+/H^+ antiporter molecules. Similarly, a study of the H^+-transport properties of *H. jubatum* root plasma membranes seems warranted especially in the context of the superior root growth and ion relations for salt stressed plants (see Chapter 3.0). Our long term goal then is to correlate growth and ion accumulation of salt stressed barley roots with the ion transport properties of the various subcellular membranes.

4.5 Summary

The H^+-transport and surface potential properties of plasma membrane vesicles isolated from salt stressed Harrington root tissue were examined using pH-sensitive (quinacrine) and potential-sensitive (ANS) fluorescent dyes. Membrane vesicles prepared from roots that had been salinized at a Ca/TC = 0.02 (EC = 0.90 S/m) had less H^+-transport activity than vesicles from roots salinized at a higher Ca/TC of 0.07 (EC = 0.96). There was evidence of Na^+/H^+ exchange activity in both of the plasma membrane fractions, however this activity appeared to be greater in membranes obtained from roots stressed at Ca/TC = 0.02. The decrease in H^+-pump activity may be detrimental to root growth because Na^+ extrusion from the cell via the Na^+/H^+ antiporter is driven by the H^+-gradient generated by the plasma membrane-bound ATPase. A smaller H^+-gradient between the cell exterior and the cytoplasm would result in a decreased potential for Na^+ extrusion. This diminished H^+-pump activity may be partially compensated for by an apparently greater amount of Na^+/H^+ antiporter activity in these same membranes. Another important membrane property, the membrane surface potential, was not altered by salinity stress. The results suggest that salinity alters certain properties of the barley root plasma membrane that are fundamental to ion transport. These same properties are influenced by Ca/TC ratio of the stress solution.

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5.0 RESPONSE OF BARLEY SEEDLINGS TO SALINITY AND CALCIUM AVAILABILITY

5.1 Objectives

A series of laboratory experiments were carried out to determine the importance of interactions between calcium supply and excess salinity in controlling membrane integrity and growth of barley seedling roots.

5.2 Methods and Materials

The methods and varieties used in these experiments are described in the following under experiment code numbers. These codes will be referred to when reviewing and interpreting the results. In all cases data were analyzed using analysis of variance.

1BAR8702

Three varieties of barley including Bonanza, California Mariout and Harrington were exposed to the following treatment solutions:

(1) distilled water (control)
(2) 83 mmol Mg as $MgSO_4$
(3) 83 mmol Mg as $MgSO_4$ plus 9 mmol Ca as $CaCl_2$
(4) 83 mmol Mg as $MgSO_4$ plus 1.8 mmol Ca as $CaCl_2$

Seeds were germinated in distilled water on filter paper in petri dishes. After one day seedlings were transferred to styrofoam flotation trays in plastic containers with 500 ml of test solution.

Roots of three-day old seedlings were cut off, rinsed with distilled water and transferred to Erlenmeyer flasks containing distilled water for measurement of the relative leakage ratio. Flasks were placed on a rotating shaker for 24 h, after which a 2.5 ml aliquot was removed for determination of UV absorbance at 280 nm (A_{280}) using a Beckman DU-8 spectrophotometer. This aliquot was replaced, and the tissues were killed by freezing the flasks at -80°C for at least one hour. Flasks were then thawed and placed on a rotating shaker at room temperature for 2-3 h, after which a 2.5 ml aliquot was removed for an absorbance measurement (A'_{280}). The relative leakage ratio (RLR) was calculated as:

$$RLR = A_{280}/A'_{280}.$$

2BAR8802

Seedlings of barley varieties Bonanza and Harrington were given the same treatments as above, except that seedlings were exposed to treatment solutions (10 ml) in individual test tubes (95 mm length, 16 mm diameter). Seeds were germinated in petri dishes on filter paper saturated with distilled water and transferred to the test tubes when two days old. After three days exposure to the treatments root lengths were measured, and RLR was determined using the technique described above.

In the case of test tube culture, root tissue was not transferred to Erlenmeyer flasks for measurement of leakage. Instead the test tube itself was used as the test vessel.

3BAR8803

Seeds of Bonanza and Harrington were germinated in distilled water and grown for two days before transfer to the following treatment solutions in individual test tubes:

(1) distilled water (control)

(2) 113 mmol Na as Na_2SO_4

(3) 100 mmol Mg as $MgSO_4$

(4) 56 mmol Na + 50 mmol Mg as Na_2SO_4 and $MgSO_4$

(5) 56 mmol Na + 50 mmol Mg as Na_2SO_4 and $MgSO_4$ + 13.4 mmol K as KCl

(6) 56 mmol Na + 50 mmol Mg as Na_2SO_4 and $MgSO_4$ + 9 mmol Ca as $CaCl_2$

(7) 56 mmol Na + 50 mmol Mg as Na_2SO_4 and $MgSO_4$ + 13 mmol K + 9 mmol Ca

After three days exposure, aliquots of solution were removed to determine A_{280} and A'_{280} using the standard technique described earlier. A duplicate set of treatments were used to measure seedling root length after three days exposure to the treatment solutions (five-day old seedlings).

4BAR8701

In this experiment seedling root growth was measured for six barley varieties (Bonanza, Harrington, California Mariout, Johnston, Beacon and CI3552) exposed to:

(1) distilled water (control)

(2) 83 mmol Mg as $MgSO_4$

(3) 83 mmol Mg as $MgSO_4$ + 9 mmol Ca as $CaCl_2$

Twenty five seeds were placed in each of four replicate 9 cm petri dishes with filter paper discs saturated with 6 ml of test solution. Mean maximum root length for each replicate was measured after two days.

5BAR8805

Four barley varieties (Bonanza, Harrington, California Mariout and Samson) were grown for 21 days using the hydroponics system described in detail in Chapter 3. Salinization of solutions was done gradually over a period of 168 h after transfer of seedlings into the growth containers. The test solutions were:

(1) standard nutrient solution (sns)
(2) sns + 50 mmol Na + 50 mmol Mg + 20 mmol Ca
(3) sns + 50 mmol Na + 50 mmol Mg + 10 mmol Ca
(2) sns + 50 mmol Na + 50 mmol Mg + 2.5 mmol Ca

Several plant parameters including number of roots, maximum root length, number of leaves, leaf weight and root weight were determined at the end of the 21 day period. Only the last two measures are discussed in this report.

5.3 Results and Discussion

5.3.1 Relative leakage ratio

Treatment of barley seedling roots with 83 mmol of $MgSO_4$ caused significant increase in the relative leakage ratio (RLR) (Table 5.1). Differences among varieties in the effect of salt tended to be small or not statistically significant. The addition of Ca to the $MgSO_4$ treatment solutions caused a significant reduction in leakage, indicating the protective role

Table 5.1 Mean relative leakage ration in four experiments on seedling roots of barley varieties Bonanza (B), Harrington (H) and California Mariout (CM).

Treatment				Variety and Experiment						
Na	Mg	Ca	K	1BAR8702			2BAR8802		3BAR8803	
	mmol			B	H	CM	B	H	B	H
Control				0.30	0.40	0.37	0.57	0.62	0.40	0.41
113	-	-	-	-	-	-	-	-	0.57	0.74
-	100	-	-	-	-	-	-	-	0.76	0.63
-	83	-	-	0.37	0.44	0.36	1.04	0.88	-	-
-	83	9	-	0.20	0.28	0.30	0.61	0.75	-	-
-	83	18	-	0.25	0.29	0.24	0.77	0.72	-	-
56	50	-	-	-	-	-	-	-	0.57	0.77
56	50	9	-	-	-	-	-	-	0.51	0.73
56	50	9	13	-	-	-	-	-	0.82	0.78
56	50	-	13	-	-	-	-	-	0.56	0.73

of calcium, even when present in concentrations as low as 9 mmol. Doubling the calcium level did not decrease leakage any further. The protective effect of calcium was less in mixed Na_2SO_4 and $MgSO_4$ salt treatments.

The relatively high RLR levels in the control solutions (distilled water) indicate that roots have a tendency to have leaky membranes, even in the absence of salt. In one experiment (Table 5.1) the leakage in Ca supplemented salt solutions was actually lower than the control. We suspect that natural variation in background Ca concentrations in barley seeds could confound the effects of the treatment solutions, and might also explain the relatively large variability among measurements of leakage from roots grown from different varieties and different seed lots within a variety.

5.3.2 Root length in barley seedlings

Salt treatments caused a significant reduction in root growth of both Bonanza and Harrington barley. The effect was strongest with $MgSO_4$ (83 mmol Mg), which reduced total seedling root length by 74% in Bonanza and 69% in Harrington (experiment 2BAR8802, Table 5.2). The effect of salt treatment was somewhat less, but still significant in the other two experiments summarized in Table 5.2.

The protective effect of calcium was apparent, particularly in seedlings treated with $MgSO_4$ alone. For example, treatment with 83 mmol Mg + 9 mmol Ca increased root length to values approaching that of the control (Table 5.2, and Figure 5.1).

Table 5.2 Mean seedling root length (mm) in barley varieties Bonanza (B), Harrington (H), California Mariout (CM), Johnston, (J), Beacon (BE) and CI3552 (CI) in three experiments.

Treatment				Variety and Experiment									
Na	Mg	Ca	K	2BAR8802		3BAR8803		4BAR8701					
	mmol			B	H	B	H	B	H	CM	J	BE	CI
Control				152	124	149	259	16	22	16	13	18	13
113	-	-	-	-	-	92	90	-	-	-	-	-	-
-	100	-	-	-	-	68	88	-	-	-	-	-	-
-	83	-	-	39	38	-	-	9	9	9	8	11	9
-	83	9	-	118	172	-	-	10	12	13	9	13	10
-	83	18	-	135	156	-	-	-	-	-	-	-	-
56	50	-	-	-	-	101	101	-	-	-	-	-	-
56	50	9	-	-	-	103	103	-	-	-	-	-	-
56	50	9	13	-	-	186	241	-	-	-	-	-	-
56	50	-	13	-	-	206	203	-	-	-	-	-	-

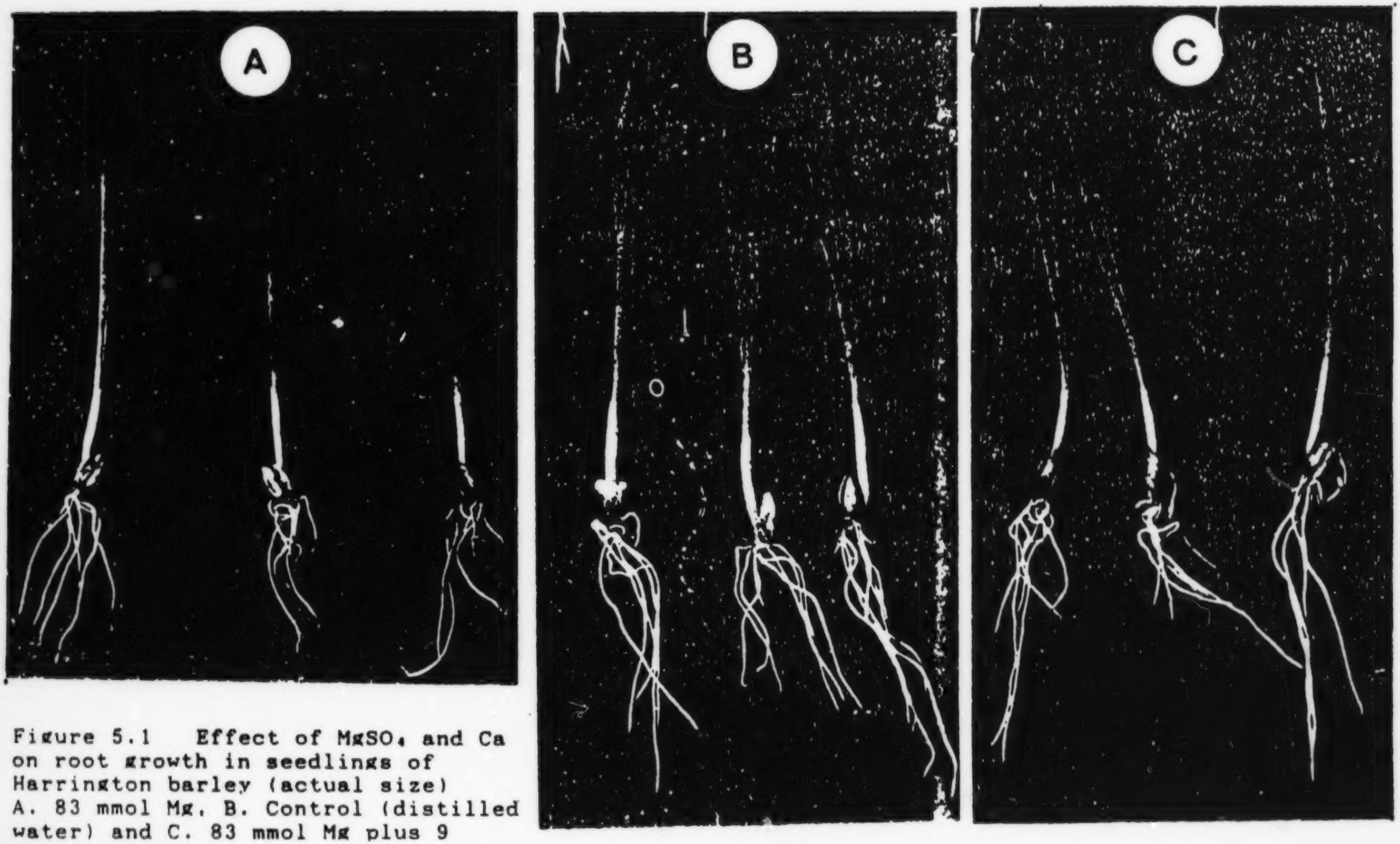


Figure 5.1 Effect of $MgSO_4$ and Ca on root growth in seedlings of Harrington barley (actual size) A. 83 mmol Mg, B. Control (distilled water) and C. 83 mmol Mg plus 9 mmol Ca as $CaCl_2$.

Effects of Ca supplementation in mixed Na_2SO_4 and $MgSO_4$ treatments were less apparent (experiment 3BAR8803). In this case, K supplement appeared to produce an effect greater than that of Ca alone. In research results discussed earlier, we showed that a key effect of Ca was in increasing K/Na selectivity. By adding K to the medium around the seedling roots we probably increased K availability to the roots, and thereby reduced the relative effect of supplemental calcium.

There were significant differences among barley varieties in their response to Mg salinity stress, and their response to supplemental calcium. The data in Table 5.2 are mean lengths of the longest seedling root, a parameter which is less responsive than total root length measured in the other experiments. Averaged over varieties, root lengths were 16.4 mm (control), 9.1 mm (83 mmol Mg) and 11.1 mm (83 mmol Mg + 9 mmol Ca). Differences among varieties might be controlled by background Ca content of seed lots as well as by differences in basic salt tolerance, although this hypothesis must be tested.

5.3.3 Root and shoot dry matter production in three-week old barley seedlings

Calcium availability reduced the weights of roots and shoots exposed to mixed Na_2SO_4 and $MgSO_4$ salts. This effect was summarized by calculating the slope of the linear regression between root or shoot weight, and the log transformation of the total cation/calcium ratio.

The results (Table 5.3) show that decreasing calcium

Table 5.3 Response of root and shoot dry matter production to decreased calcium availability in four barley varieties grown in solution culture for three weeks. Values are the scopes and y-intercepts from the linear regression analysis.

Variety	Root Production		Shoot Production	
	slope	y-intercept	slope	y-intercept
Bonanza	-25	50	-24	86
Harrington	-27	67	-20	106
Samson	-26	63	-	-
California Mariout	-42	93	-42	147

availability actually reduced growth of roots in variety California Mariout more than the other varieties. The effect on shoot growth was similar. Despite this greater reduction, California Mariout still had a high yield under the high salinity, low calcium regime, because it also has a larger root system under low salt stress. That is, yield decreased more, but from a higher initial value. This is reflected in the larger y-intercept value for this variety. Harrington showed a similar trend, particularly for shoot production.

5.4 Summary

Salt stress causes membrane damage to barley roots leading to increased leakage of UV-absorbing compounds from the cells. The effect of $MgSO_4$ is greater than that of Na_2SO_4. Increased availability of calcium reduces the amount of membrane damage. The length of seedling roots is reduced by salt stress, but relatively low levels of Ca can overcome at least some of the adverse effects of salts on root growth. Large differences in response of barley varieties to excess salt or to low calcium were not observed. In fact, a variety generally considered to be highly salt tolerant (California Mariout) was relatively sensitive to reduced Ca availability under salt stress, but still produced substantial dry matter under salt stress because of its vigorous growth. The Saskatchewan variety Harrington shows a similar, but less pronounced response to salinity and low calcium stress, suggesting that productive varieties are inherently more "salt tolerant", defined in terms of yield under salt stress.

6.0 CALCIUM AMENDMENTS IN FIELD PLOTS

6.1 Introduction

Results of our earlier research led us to hypothesize that supplemental calcium could be effective in improving the growth of barley growing on saline soils in the field. To test this hypothesis, we set up experimental plots along a gradient of soil salinity at a site on the Goodale Research Farm. The objective of the work in 1989 was to determine the effect of calcium chloride soil amendments on growth and ion relations of barley and on soil chemical properties. Calcium chloride was selected because it is a highly soluble form of calcium, and is relatively inexpensive and readily available.

We intend to continue this experiment in 1990, therefore the material presented in this chaper is essentially a progress report based on one-year of data. Several analyses remain to be done, and the results should be considered tentative.

6.2 Methods and Materials

A field trial of the effects of calcium soil amendments was conducted at the Goodale Research Farm during 1989. A preliminary salinity survey of the site was done using ane EM-38 portable conductivity meter. Four blocks of differing conductivity were selected over a range from block 1 (high conductivity) to block 4 (low conductivity). The blocks were subdivided into four rows of three plots, each measuring 2 x 5 m with a one m buffer strip between plots. Calcium chloride was applied to the plots at three levels:

Treatment 1	0 $CaCl_2$ (control)	
Treatment 2	1.9 kg (77% $CaCl_2$)	0.146 kg/m^{-2}
Treatment 3	5.2 kg (77% $CaCl_2$)	0.400 kg/m^{-2}

Every row had each of the three treatments in random arrangement. The $CaCl_2$ was hand applied and shallow incorporation was done immediately.

Harrington barley was seeded at 100 lb/acre along with 50 lb/acre of 11-51-0 fertilizer using a hoe drill. Moisture content of the soil was sufficient for germination at seeding and rainfall followed shortly thereafter in amounts sufficient to incorporate the $CaCl_2$.

Crop emergence began on 15 May. On 19 May, the following emergence percentages were recorded:

Block 1	<10%
Block 2	30-40%
Block 3	50-60%
Block 4	60-70%

Emergence counts were done on 2 June 1989, using a 0.5 m x 0.5 m quadrat which was randomly located in the plots. In all cases, three different rows were counted. The emergence was found to be:

Block 1	20 per 0.25 m^2
Block 2	31 per 0.25 m^2
Block 3	45 per 0.25 m^2
Block 4	54 per 0.25 m^2

Plant growth and development was monitored weekly from 2 June to 19 July at which time approximately 80% of the heads had emerged. The technique used is described in *Visual Quantification of Wheat Development* by J.R. Haun. As well, canopy heights were measured weekly from 29 June to 26 July.

Green plant material was harvested from 1 m^2 quadrats in each plot on 27-28 July for growth measurements and specific ion analysis. The 1 m^2 samples were taken from the east end of each plot with the first row of plants and 0.5 m from each side of the plot excluded. Ten plants were subsampled from this harvest, and the number of viable tillers, plant height, and the number of heads per plant were recorded. The remaining plants were then counted and broken down into leaves, stems and heads and a complete analysis was run by the University of Saskatchewan Soil Testing Laboratory. Chloride is currently being analyzed at the United States Salinity Laboratory, Riverside, California.

Blocks three and four matured about 16-17 August and Blocks one and two on 29-30 August, 1989. At maturity a 35 ft^2 area was harvested from each plot at ground level and 50 heads were randomly selected. The plants were dried, weighed, threshed and the weights of plants and grain were determined. The 50 head samples were threshed, weighed and counted to determine the number of kernels per head and 1000 kernel weights.

After the harvest, soil samples were taken from each plot in depth increments of 0-15 cm, 15-30 cm, 30-60 cm and 60-100 cm. A complete salinity analysis was run on these samples by the University of Saskatchewan Soil Testing Laboratory.

6.3 Results and Discussion

The background salinity, based on electrical conductivity (EC) measurements ranged from 1.6 dS m^{-1} (block 4) to 16.2 dS m^{-1} (block 1), providing a wide gradient for the testing of effects of salinity and calcium amendments of soil (Table 6.1). A complete analysis of soluble cations and anions in soil layers to a depth of 100 cm was carried out, but data have not been included here.

Calcium amendment raised the Ca concentration in the soil solution from 8.5 to 11.2 and 18.5 mmol L^{-1} (0-15 cm), when averaged across blocks (Table 6.1). The higher the background salinity, the smaller the increase in soluble calcium. The treatment raised the Ca/Total Cation ratio by over 3 times in the most saline plots (block 1).

Grain yield was significantly reduced as salinity increased from block 4 to block 1 (Table 6.2). Yield on the most saline block was only about one third of the control (non-saline) block. Differences in crop development among blocks were very apparent (Figure 6.1). The effect of Ca amendments on grain yield was not statistically significant.

Yield components were affected by overall salinity, but there were no significant differences among calcium treatments. Salinity greatly reduced the number of kernels per head (Figure 6.1, Table 6.2), but had relatively less effect on 1000 kernel weight. Other studies have shown kernel size to be a conservative characteristic, that is, the number of kernels per head is reduced more, proportionally, than kernel size.

Table 6.1 Effect of calcium chloride treatments on soil chemical characteristics in 0-15 soil layer at Goodale site in 1989. Ion concentrations are mmol L^{-1} in saturation extract. Treatments included the control (Ca0), low calcium (Ca1) and high calcium (Ca2).

Block	Conductivity (dS m^{-1})			Calcium (mmol L^{-1})			Ca/Total Cations		
	Ca0	Ca1	Ca2	Ca0	Ca1	Ca2	Ca0	Ca1	Ca2
1	16.2	11.3	14.3	14.0	15.6	20.0	0.08	0.23	0.29
2	8.8	9.3	13.4	13.6	17.0	19.4	0.19	0.31	0.20
3	2.3	4.5	6.3	5.0	9.3	17.7	0.37	0.45	0.70
4	1.6	2.3	5.6	3.9	5.5	17.3	0.52	0.64	1.10
Mean	6.5	6.3	9.3	8.5	11.2	18.5	0.31	0.43	0.62

Table 6.2 Grain yields and yield components of Harrington barley in Goodale Field Site (1989). Blocks were set up along a salinity gradient.

Block	Salinity EC (dS m^{-1})		Grain Yield		Yield Components	
	0-15 cm	0-100cm	kg ha^{-1}	bu ac^{-1}	kernels/ head	1000 kernel weight (g)
1	13.9	12.3	888	17	12.9a[1]	39.9a
2	10.5	9.9	1341	25	15.0b	44.8b
3	4.4	7.3	1513	29	15.3b	42.9b
4	3.2	7.9	2789	53	20.1c	44.1b

[1] Values not followed by the same letter are significantly different at the 0.05 level.

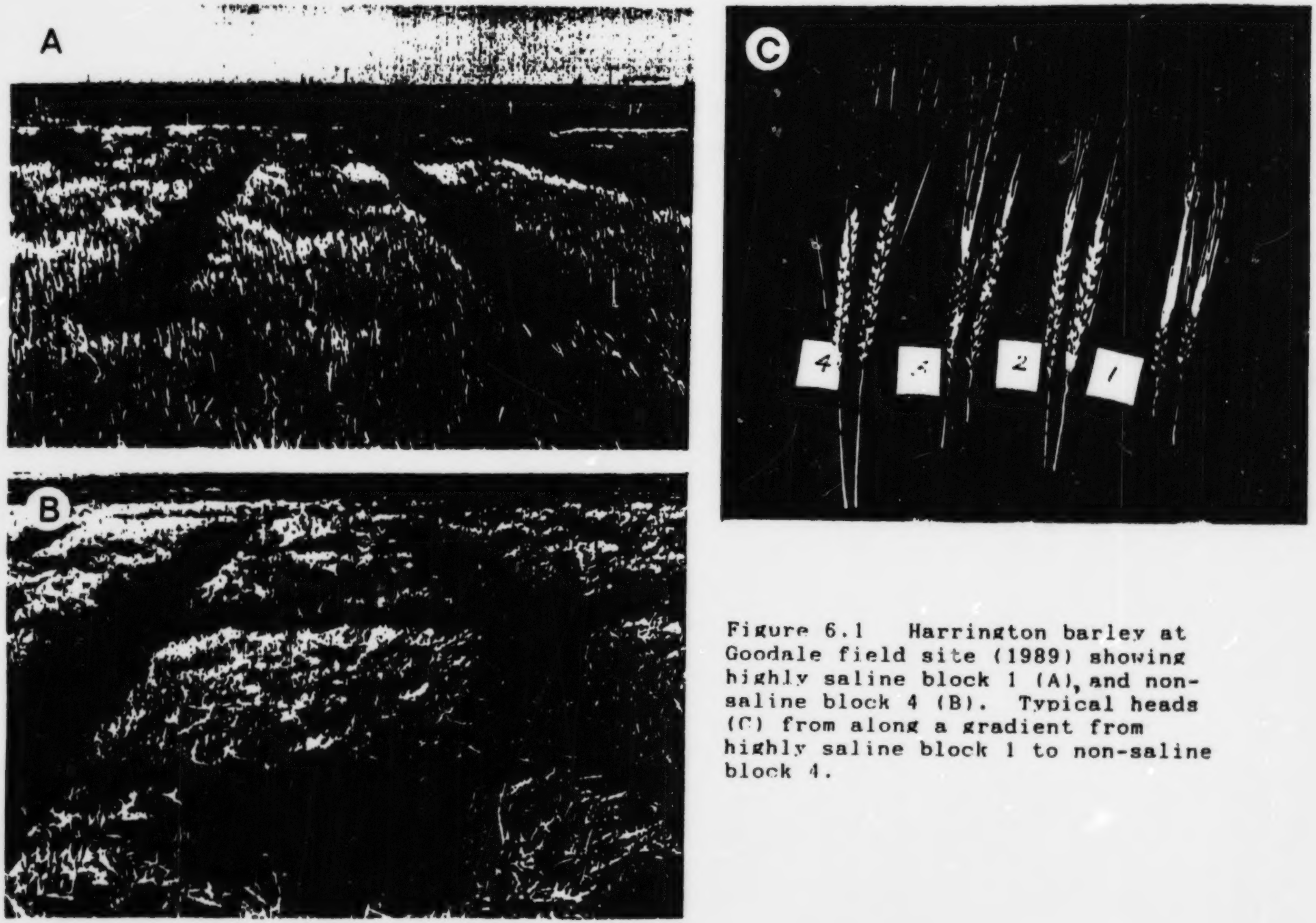


Figure 6.1 Harrington barley at Goodale field site (1989) showing highly saline block 1 (A), and non-saline block 4 (B). Typical heads (C) from along a gradient from highly saline block 1 to non-saline block 4.

Plant height was unaffected by calcium amendment, but was reduced by the relatively high salinities in blocks 1-3 (Table 6.3). Both salinity and calcium amendments reduced plant growth on an area (m^{-2}) basis, as measured by number of plants, number of heads, and leaf and stem weights. Despite this, there was no significant difference in head weight per unit area. In some way calcium-treated plants appear to compensate for loss of vegetative vigour, so that head weight and grain yield (discussed earlier) remain constant.

Plant development over the growing season was evaluated using the Haun Scale. Analysis of variance revealed a significant effect of calcium amendment on development: plants on calcium treated plots had slightly fewer leaves than control plants (mean Haun stage 6.01 and 6.14 for treated plants, and 6.29 for control plants). The block (or salinity) effect was greater. The average Haun stage in block 1 was 5.54, significantly lower than 6.45 in block 4. There was a significant block *x* date interaction, showing that development proceeded more slowly in the highly saline area, compared to the control. This difference was most apparent early in the season, when plants in non-saline plots were as much as 1.5 Haun scale units more advanced than those in highly saline plots. By late season, all blocks and calcium treatments had reached about the same Haun stage of development.

Calcium amendments produced significant effects on the inorganic ion composition of leaves, stems and heads of barley. Calcium treatments were particularly effective in reducing the Na concentration in leaves of plants growing on the most saline

Table 6.3 Plant growth parameters determined from m² harvests of Harrington barley at Goodale field site on 27 July 1989. Blocks were set up along a salinity gradient. Differences among calcium amendments are shown only when statistically significant, based on analysis of variance.

Block	Plant Height (cm)	Number of Plants per m²			Number of heads per m²		
		Ca0	Ca1	Ca2	Ca0	Ca1	Ca2
1	44	90	84	77	342	307	327
2	49	113	85	81	755	380	501
3	49	116	118	91	541	481	408
4	64	174	176	126	722	616	551

Block	Leaf Weight g m⁻²			Stem Weight g m⁻²			Head Weight g m⁻²
	Ca0	Ca1	Ca2	Ca0	Ca1	Ca2	
1	42	44	38	121	122	99	126
2	73	62	43	190	166	134	216
3	72	76	43	158	163	119	220
4	111	96	72	243	228	209	453

plots. Averaged over blocks, the Na content of leaves dropped from 21.5 mmol/100 g in the control, to 11.2 mmol/100 g in the high calcium amendment. This was paralleled by a doubling of the K/Na ratio. The Ca content of leaves increased slightly with calcium amendment; Mg and K remained relatively constant across all calcium amendments.

Stem tissue of barley contained much less Ca, somewhat less Mg, but higher levels of Na and K than leaves. The effects of calcium treatment were not apparent.

Calcium treatment also produced little effect on the ionic composition of barley heads. Heads were distinctive in that all major cations except K tended to be excluded. The K/Na ratios in heads were up to 5 times greater than for leaves and stems.

6.4 Summary

Soil amendment with calcium chloride increased the concentration of Ca in the saturation extract of the 0-15 cm soil depth. The ratio Ca/Total Cations also was increased by the treatment. There were large differences in salinity among blocks along the natural salinity gradient at the research site. Calcium treatments had no significant effect on grain yield or yield components (kernels/head, 1000 kernel weight), but salinity substantially reduced these parameters. Calcium treatments decreased vegetative growth per unit area (number of plants, leaf weight and stem weight), but did not decrease head weight. We conclude that calcium amendments in 1989 had a negative effect on vegetative yield, but no effect on grain yield. The effects of calcium treatments on cation content of plant tissue were positive, that is, supplemental calcium decreased Na (a potentially harmful ion), increased the K/Na ratio, and increased Ca content. We are not sure, as yet, why these positive effects were not translated into improved vegetative and grain yields. It is possible that excess chloride in $CaCl_2$ treated plots may have accumulated in plant tissues, overriding the positive effects of calcium. Tissue samples are being analyzed for Cl content, but the results are not available at this time.

7.0 THE MEMBRANE LEAKAGE TEST AS A MEASURE OF COMPARATIVE SALT TOLERANCE IN BARLEY VARIETIES

7.1 Introduction

Environmental stresses including freezing, drought and salinity are known to injure plants by damaging cellular membranes, allowing cellular constituents to leak out. Membranes lose their ability to function properly, which causes disruption in plant growth and physiology. A commonly used test of membrane damage is based on measurement of the amount of leakage of cellular constituents into a test solution containing plant tissues such as root segments or leaf discs (Redmann *et al.* 1986).

The objective of the research reported here was to use the relative leakage ratio (RLR) method to compare the responses of barley varieties to experimental salt stress. Leakage from leaf, coleoptile and root tissues was compared in up to 30 varieties or lines of barley supplied by barley breeders in the Department of Crop Science and Plant Ecology.

7.2 Methods and Materials

The RLR method was used to test the leakage from field grown leaf tissue (Part 1), seedling coleoptiles (Part 2), and field grown root and leaf tissue (Part 3).

Part 1

Thirty varieties (or lines) of barley were grown in rows in field plots at the University of Saskatchewan Preston Plots. For each variety 10-20 of the youngest fully-emerged leaves were removed from several plants during the period 15 July-26 July, 1985. Discs (7 mm diameter) were cut from these leaves and placed in test solutions including (1) distilled water (control) and (2) 0.5 mol Na_2SO_4. The RLR was determined and adjusted for background leakage using the technique of Flint (1967).

Part 2

Seeds of the same thirty varieties were germinated in petri dishes on filter paper discs wetted with 6 ml of distilled water. After four days, 20 coleoptile segments (0.5-1 cm long) were cut off the seedlings and used for each replicate. Tissues were exposed to the following treatments: (1) distilled water (control), and (2) 0.05 mol Na_2SO_4. Appropriate concentration of the test solution had been determined in preliminary tests. RLR was then determined, and a correction made for background leakage.

Part 3

Whole plants of 12 barley varieties, with roots and soil attached were dug up from field plots on 8-11 July, 1986. Twenty leaf discs were cut from the youngest fully-emerged leaves of four plants (replications). Soil was washed off the roots, and 7 mm root segments were cut off for testing of RLR. Leaf and root tissue samples were rinsed with distilled water for about one hour, and then exposed to treatment solutions (15 ml) in Erlenmeyer flasks for 24 hours. Test solution included: (1) control (distilled water), and (2) 0.5 mol Na_2SO_4 (roots) or 2.0 mol (leaf discs). RLR was then determined using the standard technique.

7.3 Results and Discussion

There were highly significant differences among RLR values for barley leaf and coleoptile tissue exposed to Na_2SO_4 salt stress. Values for coleoptiles ranged from 0.10 (indicating low leakage, and tolerance to salt) in variety Forrest, to 0.58 (high leakage, and presumed membrane damage) in variety Scout. RLR values for the leaves of the same varieties were substantially lower than for the coleoptiles (0.07 to 0.20), indicating that field-grown leaves are apparently much more tolerant of salt than laboratory-grown coleoptiles.

Experiments on the protective effect of calcium (Chapter 5) showed that Ca reduces membrane leakage. We hypothesize that barley leaves, which have a relatively high Ca content, have less leakage than coleoptiles when treated with salt. Also, the variation among barley varieties in coleoptile leakage could be due to differences in Ca content of seed lots, rather than to salt tolerance *per se*.

There was a poor relationship between leakage of coleoptiles and leaves in the 1985 experiments (correlation coefficient -0.463). The reliability of this test as a measure of salt tolerance is questionable until it is correlated with some independent measure of salt tolerance.

Results of the comparison of leakage from roots and leaves in 1986 also were not promising. Analysis of variance showed that salt treatment significantly increased the RLR of leaf discs, but there were no significant differences in RLR among the 12 varieties tested. There were no differences in RLR of roots among the barley varieties. Leakage was high in both the control and salt-treated roots (RLR of 0.64 and 0.62, respectively). Root tissue appears to be particularly sensitive to any experimental manipulation, and therefore a great deal of caution is required in measuring RLR of roots.

7.4 Summary

The relative leakage ratio (RLR) technique was used to compare resistance to salt treatment in leaves, coleoptiles and roots of barley. In some experiments there were significant differences in RLR among varieties. The results were inconsistent, and apparently strongly dependent on experimental conditions, pre-conditioning of plants and the plant part being tested. Based on our results we are reluctant to recommend this approach for evaluating comparative salt tolerance of different varieties unless a better experimental protocol can be developed, and the relationship between leakage and overall salt tolerance of whole plants can be proven.

7.5 References

Flint, H.L., B.R.Boyce and D.J. Beattie. 1967. Index of injury - a useful expression of freezing injury to plant tissue as determined by the electrolytic method. Can. J. Plant Sci. 47:229-230.

Redmann, R.E., J. Haraldson and L.V. Gusta. 1986. Leakage of UV-absorbing substances as a measure of salt injury in leaf tissue of woody species. Physiol. Plant. 67:87-91.

8.0 CONCLUSIONS AND FUTURE RESEARCH

This study documents the significant differences in growth response, ion relations, and calcium utilization, that are observed between cultivars of wheat, barley, and wild barley (*Hordeum jubatum*) species subjected to a complex salinity stress. The following relative salt tolerance rating can be made for the plants used in this study, *Hordeum jubatum* 'foxtail barley'> *H. vulgare* cv. Abee, Harrington, Bonanza> *Triticum aestivum* cv. Neepawa, which is based upon plant dry matter production measured against the calcium/total cation ratio (Ca/TC) of salts in solution culture experiments.

Our data suggest that the efficient use of available calcium under saline growing conditions was a primary factor in determining whole plant response to salinity. The inherently higher levels of root calcium maintained by *H. jubatum* plants and the greater amount of calcium translocated from root to shoot may be a primary factor in the observed salt tolerance of this species as compared to commercial cultivars. *H. jubatum* plants appear to have more efficient calcium uptake, compartmentation, and translocation mechanisms that may be central to the maintenence of normal root growth under saline conditions. This superior root growth under stress conditions may substantially contribute to the greater whole plant tolerance to salt stress observed with foxtail barley. In the commercial barley cultivars the presence of a higher ratio of calcium:total cations in the salinized medium, and not the actual level of calcium in root tissue, improved root growth. Calcium also had a strong positive influence on the K,Na selectivity of these roots. These results suggest that the presence of calcium in the root cell wall apoplastic space, or at the outside face of the root cell plasma membrane may play a key role in the salinity tolerance of commercial barley lines.

Membrane ion permeability differences between *H. jubatum* and commercial barley lines may be closely linked to salt tolerance. The greater K,Na selectivity of *H. jubatum* roots and shoots under salt stress, as compared to cultivated barley lines, reflect differences in membrane physical properties or membrane mediated ion transport phenomena. In Harrington root cell plasma membranes, salinity stress under low Ca/TC conditions decreases the salt stimulated activity of the H^+-pumping ATPase. This enzyme maintains the electrochemical gradient that provides the energy for the transport of essential ions from the apoplastic space into the cytoplasm and also for the extrusion of Na from the cell. Our results indicate the presence of a Na^+/H^+ antiporter on the root cell plasma membranes of this cultivar which may be one of a series of integrated ion transport mechanisms coordinated to maintain favorable K,Na selectivities in root tissue. These results coupled with the observed calcium mediated reductions of salt-induced ion leakage suggest that calcium ions closely interact with structural and functional components of the membrane that are involved with permeability and transport.

Preliminary data from our field study on the influence of soil incorporated calcium chloride amendments on the salinity tolerance of Harrington barley are providing mixed results. While soil calcium additions positively influenced ion relations and K/Na ratios in plant tissues, added calcium did not improve grain yield above the levels harvested from untreated saline plots. Vegetative yield was decreased with calcium addition relative to unamended plots. Based upon these results, and in comparison with our solution culture experiments, we believe that there is a reasonable liklihood that elevated soil chloride levels, resulting from the amendments, may be offsetting any beneficial effects of added calcium.

Taken together, the results of this study on the physiology of salt tolerance in crop plants provides us with several new directions in our research program. Clearly, we need to make use of the inherent salt tolerance of *H. jubatum* germplasm. A further characterization of the whole plant and cell physiological properties of *H. jubatum* plants that provide this species with superior salt tolerance as compared to *H. vulgare* cultivars seems warranted. As a first step, we need to identify those functional and structural differences at the cellular, tissue, and organ levels that account for the differences in calcium and salt accumulation observed in tissues of commercial barley lines and the wild species. In this respect the effect of salinity on the function and structure of three critical membranes in *H. jubatum* and Harrington roots needs to be examined: the root cell plasma membrane, the tonoplast (vacuole membrane), and the xylem parenchyma/xylem vessel interface. Some initial work with Harrington root plasma membranes has been presented in this report. We have recently initiated a comparative study of salinity-induced changes in the protein composition of Harrington and *H. jubatum* root and crown tissue with the intention of identifying those proteins that may be associated with salinity tolerance.

Secondly, we need to know more about the possible variability of salt tolerance between different ecotypes of *H. jubatum*. Specifically, do plants of this wild species that inhabit saline sites differing in salt composition possess similar or different types and levels of salinity tolerance.

Finally, we need to clarify the initial results of our first year field studies with additional work on different types of calcium amendments. We hope to define what role, if any, anions might have in mediating whole plant salt tolerance.

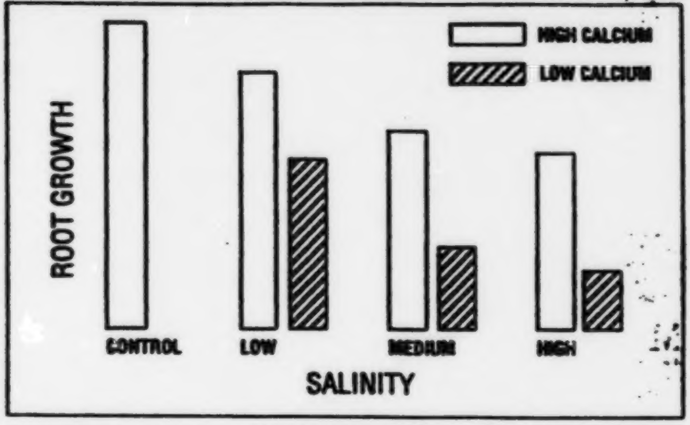
A comprehensive study including whole plant response to salinity under field and growth chamber conditions, tissue ion relations, and stress-induced changes in cell physiology will provide us with an integrated picture of the mechanisms by which salinity reduces plant growth and yield.

Salt tolerance in barley

Several million hectares of land in the prairie provinces are affected to some degree by soil salinity. On a global basis, soil salinity affects as much as 950 million hectares. For the most part soil salinization is a natural geological process, but in many areas, land use practices such as summerfallowing and irrigation also contribute to the problem. Soil salinity has made extensive areas unsuitable for conventional crop production.

Salinity affects plant growth by reducing the availability of soil water and by interfering with the supply of essential mineral nutrients. In some cases plant roots are forced to grow in soil water with a salt content greater than that of seawater. Excess quantities of salt in the roots and shoots may become toxic to the plant by damaging the thin membranes which enclose each plant cell and by interfering with the enzymes which are essential for proper cell functioning.

Research on salinity problems in Saskatchewan has concentrated on the proper selection of tolerant cultivars of conventional crops, and on improving agronomic and irrigation practices. A good publication describing these approaches is "Soil Salinity - A Study in Crop Tolerances and Cropping Practices" by H.M. Holm, published by the Plant Industry Branch of Saskatchewan Agriculture.



Several plant physiologists and plant ecologists working on salt tolerance have proposed research on those plant species which have adapted naturally to saline conditions. These plants are called halophytes, a term which literally means "salt plant." Some of these plants have developed tolerance to extremely saline soils. For example, glasswort, a small succulent species which forms a reddish-colored ring of vegetation around saline sloughs is one of the most salt-tolerant plants in the world.

Although most of our halophytes are of little economic importance, a few are valuable salt-tolerant forages. Slender wheatgrass (variety Revenue) was developed from a native halophyte population in Saskatchewan. Our most salt-tolerant cereal crop, barley, has wild relatives which are even more tolerant, including wild barley (or foxtail barley), a weedy halophyte native to Saskatchewan.

Some of our recent research in the Department of Crop Science and Plant Ecology has been directed towards understanding how excess salts affect the growth of barley. We are trying to determine how barley varieties compare with wild barley, and more specifically, to identify what plant properties determine the degree of salt tolerance. We look on the wild species as "models" of very high salt tolerance from which we can learn a great deal.

The saline soils of Saskatchewan contain large quantities of sodium and magnesium sulfates. In this respect they differ from many other areas of the world which suffer from excess sodium chloride, or common table salt. One of the major findings of our research group is that excess magnesium and sodium interfere with the availability of calcium, an essential mineral nutrient in plants as well as people. Cultivated barley is sensitive to a combination of low calcium and high salinity, but wild barley is not.

One of the keys to understanding salt tolerance is in determining how plant root systems function when exposed to salty soil water. In our experiments plants are grown in solution culture, which provides the plant with nutrients along with carefully-controlled amounts of different salts. This soil-less technique is required in order to give ready access to the roots for taking measurements, and to allow more precise control of the different salts.

Roots act as the first line of defence against the penetration of the plant by excess salts. Cultivated barley roots are much more sensitive to salts than those of wild barley. The graph shows that root growth of the barley variety Harrington is greatly reduced by salinity, especially with a low calcium supply. When the calcium level in the solution culture is increased, root growth improves greatly. Roots of wild barley grow well under saline conditions, even at low calcium levels.

The sodium content of the shoot rises in barley exposed to excess salinity indicating that the root system has lost it ion selectivity.

A healthy root system is able to absorb essential minerals such as potassium, and exclude potentially harmful ones such as sodium, even when the latter is present in relatively large amounts. A viable root system is also required for the plant to use available soil water efficiently. Excess salts damage roots causing them to lose their ability to regulate uptake of nutrient and salt ions. Injured roots actually become "leaky." The sodium content of the shoot rises in barley exposed to excess salinity indicating that the root system has lost its ion selectivity.

The uptake of essential minerals is regulated by the individual cells that make up the root tissue. The cell membrane serves as the primary barrier to the free movement of ions and nutrients from the outside environment to the inside of the cell. This membrane also serves to enclose the contents of the cell. We might say that a plant cell is similar to a house. The outside walls and roof represent the plasma membrane while the rooms in the house represent compartments within the cell where ions, nutrients, and enzymes are stored. The doors and windows of a house allow people and products to enter or leave.

Similarly, "doors" and "windows" on the cell membrane, made out of proteins, are involved in the transfer of ions and nutrients in and out of the cell.

We are determining how salinity affects these transfer proteins on the membranes in root cells of commercial and wild barley. Our results to date show that the transfer proteins located on the membranes of wild barley root cells maintain their activity under salinity stress whereas in Harrington barley this activity is reduced. This suggests that one reason for the salt tolerance of wild barley may be its superior ability to regulate ion uptake under salt stress conditions. We are in the process of identifying other properties of the cell membrane that may be useful determining salt tolerance.

The results of our research could have future application in two general areas. First, by knowing which plant characteristics are essential for salt tolerance, plant breeders may be able to use these characteristics to select for varieties which grow better under salt stress. Second, a better understanding of the effect of supplemental calcium could lead to development of fertilizer practices for reducing the harmful effects of excess salts. There are several obstacles to increasing crop yield under salt stress but the existence of salt tolerant plants in nature provides promise that these obstacles can be overcome.

Bob Redmann and [illegible] are Professor and Professional Research Associate, respectively, in the Department of Crop Science and Plant Ecology, University of Saskatchewan.

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Appendix B

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Comparative Salt Tolerance of Commercial and Wild Barley Species and the Effect of Calcium. C. G. SUHAYDA*, A. L. CIPYWNYK and R. E. REDMANN, Univ. of Saskatchewan. A comparative study on the influence of calcium on the growth response of two barley species, *Hordeum vulgare* (L.) 'Harrington' and *Hordeum jubatum* 'foxtail barley', to Mg and Na sulfate based salinity stress was conducted. A differential response to salt stress was observed between these species when stressed under low Ca conditions (Mg:Na:Ca= 20:20:1) at EC values ranging from 0.12-1.88 S/m. Harrington leaf area, foliage dry weight, and root dry weight were significantly reduced by this stress. *H. jubatum* plants showed only minimal effects of salt stress and in striking contrast to Harrington plants, *H. jubatum* plants displayed superior root growth when stressed under identical conditions. Salt stress at a higher Ca level (Mg:Na:Ca=5:5:1) significantly improved the growth of Harrington plants and slightly improved the growth of *H. jubatum* plants. Ion analysis of root and leaf tissue indicated that *H. jubatum* accumulated more Ca in root and leaf tissue than Harrington. *H. jubatum* was also more efficient in excluding Na from leaf tissue than Harrington. The K:Na selectivity of root and shoot tissue of *H. jubatum* was superior to Harrington under salt stress.

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